

WEST Search History

DATE: Thursday, May 10, 2007

Hide? Set Query
Name

DB=PGPB,USPT,EPAB; PLUR=YES; OP=ADJ

- L65 L64 and (conjugat\$ or link\$ or coupl\$)
- L64 L63 not @ay>2000
- L63 L62 and antibod\$
- L62 L61 and l50
- L61 IR3 or 5C3 or MC192
- L60 L59 and (conjugat\$ or link\$ or coupl\$)
- L59 L58 and l54
- L58 L57 not @ay>2000
- L57 antibod\$ and L56
- L56 L55 and l50
- L55 L54.clm. or l54.ab. or l54.ti.
- L54 (IGF-1R) or (IGF 1R) or (IGFR) or (insulin growth factor type 1)
- L53 L52 not @ay>2000
- L52 L51 and antibod\$
- L51 L50 AND L49
- L50 (cancer\$ or tumor\$ or neoplas\$)
- L49 l44 and l47
- L48 p45 and L47
- L47 L46.ab. or l46.clm. or l46.ti.
- L46 neurotrophin receptor
- L45 neurotrophin receptor
- L44 p75
- L43 L40 and L42
- L42 L39 and L41
- L41 internal\$
- L40 dox\$ or (taxol or paclitaxel)
- L39 ("4997913"|"5084560"|"5208323"|"5258453"|"5869045"|"6020145"|"6030997"|"6140100")
- L38 L37 and L36
- L37 L21 and L29
- L36 antibod\$

- L35 L34 not @ay>2001
- L34 L33 and chemotherap\$
- L33 L31 and conjugat\$
- L32 L31 and conjugat?
- L31 L30 and antibod\$
- L30 L29.ab.
- L29 p-glycoprotein
- L28 L27 and antibod\$
- L27 (4062831 or 4097470).pn.
- L26 L24 and MDR
- L25 L24 and MRD
- L24 L23 and antibod\$
- L23 L22 and L21
- L22 doxorubicin
- L21 hpma
- L20 L18 and antibod\$
- L19 L18 and conjugat\$
- L18 L17 or L16
- L17 guillemard.in.
- L16 saragovi.in.
- L15 L14 and L13
- L14 Singh.in.
- L13 L12 and L11
- L12 antibod\$.ab.
- L11 IGF.ab.
- L10 L9.ti.
- L9 anti-igf
- L8 L7 and link\$
- L7 L6 and conjugat\$
- L6 L5 with antibod\$
- L5 IR3
- L4 MC192
- L3 L2 and L1
- L2 MDR
- L1 ADEPT

END OF SEARCH HISTORY

* * * * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007

| | | | |
|----------------------|--|------------|---------|
| => file caplus | | | |
| COST IN U.S. DOLLARS | | SINCE FILE | TOTAL |
| FULL ESTIMATED COST | | ENTRY | SESSION |
| | | 0.21 | 0.21 |

FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007
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FILE COVERS 1907 - 10 May 2007 VOL 146 ISS 20
FILE LAST UPDATED: 9 May 2007 (20070509/ED)

Effective October 17, 2005, revised CAS Information Use Policies apply. They are available for your review at:

<http://www.cas.org/infopolicy.html>

=> s p75
L1 3555 P75

=> s neurotrophin receptor
5950 NEUROTROPHIN
2953 NEUROTROPHINS
6713 NEUROTROPHIN
(NEUROTROPHIN OR NEUROTROPHINS)
698672 RECEPTOR
641112 RECEPTORS
832231 RECEPTOR
(RECEPTOR OR RECEPTORS)
L2 1651 NEUROTROPHIN RECEPTOR
(NEUROTROPHIN(W) RECEPTOR)

=> s 11 and 12
L3 806 L1 AND L2

=> s cancer or tumor or neoplas
=> s cancer? or tumor? or neoplas?
330454 CANCER?
468110 TUMOR?
491900 NEOPLAS?
L4 775765 CANCER? OR TUMOR? OR NEOPLAS?

=> s 13 adn 14
MISSING OPERATOR L3 ADN
The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> s 13 and 14
L5 108 L3 AND L4

=> s antibod?
L6 490570 ANTIBOD?

=> s 16 and 15
L7 6 L6 AND L5

=> d ibib 1-6

L7 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2002:927183 CAPLUS
DOCUMENT NUMBER: 138:29102
TITLE: High affinity ligand for p75
neurotrophin receptor
INVENTOR(S): Hempstead, Barbara L.; Lee, Ramee; Teng, Kenneth K.;
Kermani, Pouneh
PATENT ASSIGNEE(S): Cornell Research Foundation, Inc., USA
SOURCE: PCT Int. Appl., 124 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|----------|-----------------|------------|
| WO 2002096356 | A2 | 20021205 | WO 2002-US16540 | 20020524 |
| WO 2002096356 | A3 | 20060518 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
UA, UG, US, UZ, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG,
AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| CA 2447986 | A1 | 20021205 | CA 2002-2447986 | 20020524 |
| US 2003087804 | A1 | 20030508 | US 2002-155886 | 20020524 |
| EP 1575477 | A2 | 20050921 | EP 2002-729305 | 20020524 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| PRIORITY APPLN. INFO.: | | | US 2001-293823P | P 20010525 |
| | | | US 2001-305510P | P 20010713 |
| | | | WO 2002-US16540 | W 20020524 |

OTHER SOURCE(S): MARPAT 138:29102

L7 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2001:863089 CAPLUS
DOCUMENT NUMBER: 136:116439
TITLE: Neurotrophins and neurotrophin
receptors in human lung cancer
AUTHOR(S): Ricci, Alberto; Greco, Stefania; Mariotta, Salvatore;
Felici, Laura; Bronzetti, Elena; Cavazzana, Andrea;
Cardillo, Giuseppe; Amenta, Francesco; Bisetti,
Alberto; Barbolini, Giuseppe
CORPORATE SOURCE: Dipartimento di Scienze Cardiovascolari e
Respiratorie, Universita "La Sapienza", Rome, 00151,
Italy
SOURCE: American Journal of Respiratory Cell and Molecular
Biology (2001), 25(4), 439-446

PUBLISHER: CODEN: AJRBEL; ISSN: 1044-1549
American Thoracic Society
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1999:642247 CAPLUS
DOCUMENT NUMBER: 132:149912
TITLE: Expression of p75LNGFR and Trk neurotrophin
receptors in normal and neoplastic
human prostate
AUTHOR(S): Guate, J. L.; Fernandez, N.; Lanzas, J. M.; Escaf, S.;
Vega, J. A.
CORPORATE SOURCE: Servicios de Urologia Hospital San Agustin, Aviles,
Spain
SOURCE: BJU International (1999), 84(4), 495-502
CODEN: BJINFO; ISSN: 1464-4096
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:412962 CAPLUS
DOCUMENT NUMBER: 127:106248
TITLE: Immortalization and controlled in vitro
differentiation of murine multipotent neural crest
stem cells
AUTHOR(S): Rao, Mahendra S.; Anderson, David J.
CORPORATE SOURCE: Division of Biology 216-76, Howard Hughes Medical
Institute, California Institute of Technology,
Pasadena, CA, 91125, USA
SOURCE: Journal of Neurobiology (1997), 32(7), 722-746
CODEN: JNEUBZ; ISSN: 0022-3034
PUBLISHER: Wiley
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 69 THERE ARE 69 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:296246 CAPLUS
DOCUMENT NUMBER: 127:15826
TITLE: Differential regulation of two sets of mesonephric
tubules by WT-1
AUTHOR(S): Sainio, Kirsi; Hellstedt, Paavo; Kreidberg, Jordan A.;
Saxen, Lauri; Sariola, Hannu
CORPORATE SOURCE: Institute of Biotechnology, Program of Developmental
Biology, University of Helsinki, Finland
SOURCE: Development (Cambridge, United Kingdom) (1997),
124(7), 1293-1299
CODEN: DEVPED; ISSN: 0950-1991
PUBLISHER: Company of Biologists
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:260381 CAPLUS
DOCUMENT NUMBER: 126:315564

TITLE: Neurotrophins and their receptors in nerve injury and repair
AUTHOR(S): Ebadi, M.; Bashir, R. M.; Heidrick, M. L.; Hamada, F. M.; El Refaey, H.; Hamed, A.; Helal, G.; Baxi, M. D.; Cerutis, D. R.; Lassi, N. K.
CORPORATE SOURCE: Dep. Pharmacology, Univ. Nebraska College Med., Omaha, NE, 68198-6260, USA
SOURCE: Neurochemistry International (1997), 30(4/5), 347-374
CODEN: NEUIDS; ISSN: 0197-0186
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
REFERENCE COUNT: 251 THERE ARE 251 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s TRKA or neutrophilic receptor tyrosine kinase

2288 TRKA
2821 NEUTROPHILIC
698672 RECEPTOR
641112 RECEPTORS
832231 RECEPTOR
(RECEPTOR OR RECEPTORS)
159381 TYROSINE
2661 TYROSINES
159919 TYROSINE
(TYROSINE OR TYROSINES)
289994 KINASE
55981 KINASES
299105 KINASE
(KINASE OR KINASES)
0 NEUTROPHILIC RECEPTOR TYROSINE KINASE
(NEUTROPHILIC(W)RECEPTOR(W)TYROSINE(W)KINASE)

L8 2288 TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE

=> s TRKA or (neutrophilic receptor tyrosine kinase)

2288 TRKA
2821 NEUTROPHILIC
698672 RECEPTOR
641112 RECEPTORS
832231 RECEPTOR
(RECEPTOR OR RECEPTORS)
159381 TYROSINE
2661 TYROSINES
159919 TYROSINE
(TYROSINE OR TYROSINES)
289994 KINASE
55981 KINASES
299105 KINASE
(KINASE OR KINASES)
0 NEUTROPHILIC RECEPTOR TYROSINE KINASE
(NEUTROPHILIC(W)RECEPTOR(W)TYROSINE(W)KINASE)

L9 2288 TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)

=> d his

(FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007)

FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007

L1 3555 S P75
L2 1651 S NEUROTROPHIN RECEPTOR
L3 806 S L1 AND L2
L4 775765 S CANCER? OR TUMOR? OR NEOPLAS?

L5 108 S L3 AND L4
L6 490570 S ANTIBOD?
L7 6 S L6 AND L5
L8 2288 S TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE
L9 2288 S TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)

=> s 19 and 14
L10 420 L9 AND L4

=> s 110 and 16
L11 95 L10 AND L6

=> s target? and 111
526093 TARGET?
L12 21 TARGET? AND L11

=> s immunoconjugate or (conjugat? or link? or coupl?)
1074 IMMUNOCOJUGATE
2329 IMMUNOCOJUGATES
2626 IMMUNOCOJUGATE
(IMMUNOCOJUGATE OR IMMUNOCOJUGATES)
235566 CONJUGAT?
493832 LINK?
823725 COUPL?
L13 1486130 IMMUNOCOJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)

=> s 113 and 111
L14 26 L13 AND L11

=> s 114 and 112
L15 8 L14 AND L12

=> s 115 not py>2002
5237136 PY>2002
L16 1 L15 NOT PY>2002

=> d ibib

L16 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2001:125543 CAPLUS
DOCUMENT NUMBER: 134:348031
TITLE: Taxane-antibody conjugates afford potent cytotoxicity, enhanced solubility, and tumor target selectivity
AUTHOR(S): Guillemand, Veronique; Saragovi, H. Uri
CORPORATE SOURCE: Departments of Pharmacology and Therapeutics, McGill University, Montreal, QC, H3G 1Y6, Can.
SOURCE: Cancer Research (2001), 61(2), 694-699
CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER: American Association for Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s 114 not py>2001
6209673 PY>2001
L17 3 L14 NOT PY>2001

=> d ibib 1-3

L17 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2001:125543 CAPLUS

DOCUMENT NUMBER: 134:348031
TITLE: Taxane-antibody conjugates afford potent cytotoxicity, enhanced solubility, and tumor target selectivity
AUTHOR(S): Guillemard, Veronique; Saragovi, H. Uri
CORPORATE SOURCE: Departments of Pharmacology and Therapeutics, McGill University, Montreal, QC, H3G 1Y6, Can.
SOURCE: Cancer Research (2001), 61(2), 694-699
CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER: American Association for Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:872516 CAPLUS
DOCUMENT NUMBER: 124:5972
TITLE: Trk A gene expression in neuroblastoma: The clinical significance of an immunohistochemical study
AUTHOR(S): Tanaka, Takeo; Hiyama, Eiso; Sugimoto, Tohru; Sawada, Tadashi; Tanabe, Masahiro; Ida, Noriaki
CORPORATE SOURCE: Department Pediatrics, National Kure Hospital, Kure, 737, Japan
SOURCE: Cancer (New York) (1995), 76(6), 1086-95
CODEN: CANCAR; ISSN: 0008-543X
PUBLISHER: Lippincott-Raven
DOCUMENT TYPE: Journal
LANGUAGE: English

L17 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:579637 CAPLUS
DOCUMENT NUMBER: 122:307343
TITLE: Nerve growth factor as a mitogen for a pancreatic carcinoid cell line
AUTHOR(S): Bold, Richard J.; Ishizuka, Jin; Rajaraman, Srinivasan; Perez-Polo, J. Regino; Townsend, Courtney M., Jr.; Thompson, James C.
CORPORATE SOURCE: Dep. Surgery, Univ. Texas Medical Branch, Galveston, TX, USA
SOURCE: Journal of Neurochemistry (1995), 64(6), 2622-8
CODEN: JONRA9; ISSN: 0022-3042
PUBLISHER: Lippincott-Raven
DOCUMENT TYPE: Journal
LANGUAGE: English

```
=> s insulin () growth factor receptor
  202363 INSULIN
  5286 INSULINS
  202444 INSULIN
    (INSULIN OR INSULINS)
  1344531 GROWTH
  4492 GROWTHS
  1346816 GROWTH
    (GROWTH OR GROWTHS)
  1036648 FACTOR
  936905 FACTORS
  1634156 FACTOR
    (FACTOR OR FACTORS)
  698672 RECEPTOR
  641112 RECEPTORS
  832231 RECEPTOR
    (RECEPTOR OR RECEPTORS)
```

```

41650 GROWTH FACTOR RECEPTOR
    (GROWTH(W) FACTOR(W) RECEPTOR)
L18      108 INSULIN (W) GROWTH FACTOR RECEPTOR

=> s (IGF-1R) or (IGF1R) or (IGF 1R)
    28084 IGF
    2542 IGFS
    28212 IGF
        (IGF OR IGFS)
    14865 1R
    496 IGF-1R
        (IGF(W)1R)
    380 IGF1R
    28084 IGF
    2542 IGFS
    28212 IGF
        (IGF OR IGFS)
    14865 1R
    496 IGF 1R
        (IGF(W)1R)
L19      819 (IGF-1R) OR (IGF1R) OR (IGF 1R)

=> s 119 or 118
L20      914 L19 OR L18

=> d his

(FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007)

FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007
L1      3555 S P75
L2      1651 S NEUROTROPHIN RECEPTOR
L3      806 S L1 AND L2
L4      775765 S CANCER? OR TUMOR? OR NEOPLAS?
L5      108 S L3 AND L4
L6      490570 S ANTIBOD?
L7      6 S L6 AND L5
L8      2288 S TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE
L9      2288 S TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)
L10     420 S L9 AND L4
L11     95 S L10 AND L6
L12     21 S TARGET? AND L11
L13     1486130 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L14     26 S L13 AND L11
L15     8 S L14 AND L12
L16     1 S L15 NOT PY>2002
L17     3 S L14 NOT PY>2001
L18     108 S INSULIN () GROWTH FACTOR RECEPTOR
L19     819 S (IGF-1R) OR (IGF1R) OR (IGF 1R)
L20     914 S L19 OR L18

=> s type 1 and 118
    1790611 TYPE
    611407 TYPES
    2263312 TYPE
        (TYPE OR TYPES)
    9125368 1
    73341 TYPE 1
        (TYPE(W)1)
L21      7 TYPE 1 AND L18

=> s 119 and 14
L22      417 L19 AND L4

```

=> s 122 and 16
L23 83 L22 AND L6

=> s 123 and 113
L24 18 L23 AND L13

=> s 124 not py>2001
6209673 PY>2001
L25 2 L24 NOT PY>2001

=> d ibib 1-2

L25 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1999:336320 CAPLUS
DOCUMENT NUMBER: 131:128377
TITLE: Expression of the insulin-like growth factor 1 receptor (IGF-1R) in breast cancer cells: evidence for a regulatory role of dolichyl phosphate in the transition from an intracellular to an extracellular IGF-1 pathway
AUTHOR(S): Dricu, Anica; Kanter, Lena; Wang, Min; Nilsson, Gunnar; Hjertman, Magnus; Wejde, Johan; Larsson, Olle
CORPORATE SOURCE: Cellular and Molecular Tumor Pathology, CCK, R8:04, Karolinska Hospital, Stockholm, S-17176, Swed.
SOURCE: Glycobiology (1999), 9(6), 571-579
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1998:798140 CAPLUS
DOCUMENT NUMBER: 130:163531
TITLE: Expression of the insulin-like growth factor-1 receptor and its anti-apoptotic effect in malignant melanoma: a potential therapeutic target
AUTHOR(S): Kanter-Lewensohn, L.; Dricu, A.; Wang, M.; Wejde, J.; Kiessling, R.; Larsson, O.
CORPORATE SOURCE: Cellular and Molecular Tumor Pathology, CCK, R8:04, Karolinska Hospital, Stockholm, 171 76, Swed.
SOURCE: Melanoma Research (1998), 8(5), 389-397
CODEN: MREEH; ISSN: 0960-8931
PUBLISHER: Lippincott-Raven Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d kwic 1-2

L25 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2007 ACS on STN
TI Expression of the insulin-like growth factor 1 receptor (IGF-1R) in breast cancer cells: evidence for a regulatory role of dolichyl phosphate in the transition from an intracellular to an extracellular IGF-1 pathway
AB In this study we provide evidence that the low expression of IGF-1R at the cell surface of estrogen-independent breast cancer cells is due to a low rate of de novo synthesis of dolichyl phosphate. The analyses were performed on the estrogen receptor-neg. breast cancer cell line MDA231 and, in comparison, the melanoma cell line SK-MEL-2, which expresses a high number of plasma membrane-bound

IGF-1R. Whereas the MDA231 cells had little or no surface expression of IGF-1R, they expressed functional (i.e., ligand-binding) intracellular receptors. By measuring the incorporation of [³H]mevalonate into dolichyl phosphate, we could demonstrate that the rate of dolichyl phosphate synthesis was considerably lower in MDA231 cells than in SK-MEL-2 cells. Furthermore, N-linked glycosylation of the α -subunit of IGF-1R was 8-fold higher in the melanoma cells. Following addition of dolichyl phosphate to MDA231 cells, N-linked glycosylation of IGF-1R was drastically increased, which in turn was correlated to a substantial translocation of IGF-1R to the plasma membrane, as assayed by IGF-1 binding anal. and by Western blotting of plasma membrane proteins. The dolichyl phosphate-stimulated receptors were proven to be biochem. active since they exhibited autophosphorylation. Under normal conditions MDA231 cells, expressing very few IGF-1R at the cell surface, were not growth-arrested by an antibody (α IR-3) blocking the binding of IGF-1 to IGF-1R. However, after treatment with dolichyl phosphate, leading to a high cell surface expression of IGF-1R, α IR-3 efficiently blocked MDA231 cell growth. Taken together with the fact that the breast cancer cells produce IGF-1 and exhibit intracellular binding, our data suggest that the level of de novo-synthesized dolichyl phosphate may be.

ST IGF1 receptor breast cancer proliferation dolichyl phosphate
IT Phosphorylation, biological
(autophosphorylation; insulin-like growth factor 1 receptor expression
mediation by de novo synthesis of dolichyl phosphate in human
estrogen-independent breast cancer)
IT Gene
(expression; insulin-like growth factor 1 receptor expression mediation
by de novo synthesis of dolichyl phosphate in human
estrogen-independent breast cancer)
IT Cell membrane
Cell proliferation
Glycosylation
(insulin-like growth factor 1 receptor expression mediation by de novo
synthesis of dolichyl phosphate in human estrogen-independent breast
cancer)
IT Insulin-like growth factor I receptors
RL: ADV (Adverse effect, including toxicity); BOC (Biological occurrence);
BPR (Biological process); BSU (Biological study, unclassified); PRP
(Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(insulin-like growth factor 1 receptor expression mediation by de novo
synthesis of dolichyl phosphate in human estrogen-independent breast
cancer)
IT Gene, animal
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(insulin-like growth factor 1 receptor expression mediation by de novo
synthesis of dolichyl phosphate in human estrogen-independent breast
cancer)
IT Mammary gland
(neoplasm; insulin-like growth factor 1 receptor expression
mediation by de novo synthesis of dolichyl phosphate in human
estrogen-independent breast cancer)
IT 67763-96-6, IGF-1
RL: ADV (Adverse effect, including toxicity); BOC (Biological occurrence);
BPR (Biological process); BSU (Biological study, unclassified); BIOL
(Biological study); OCCU (Occurrence); PROC (Process)
(insulin-like growth factor 1 receptor expression mediation by de novo
synthesis of dolichyl phosphate in human estrogen-independent breast
cancer)
IT 12698-55-4, Dolichyl phosphate
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological
study, unclassified); MFM (Metabolic formation); BIOL (Biological study);

FORM (Formation, nonpreparative); OCCU (Occurrence); PROC (Process)
(insulin-like growth factor 1 receptor expression mediation by de novo
synthesis of dolichyl phosphate in human estrogen-independent breast
cancer)

L25 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2007 ACS on STN
AB The insulin-like growth factor-1 receptor (IGF-1R) and its possible protective effect on apoptotic cell death in malignant melanoma was analyzed in four com. melanoma cell lines. Inhibition of N-linked glycosylation by tunicamycin, which has previously been shown to block the translocation of IGF-1R to the cell surface, blocked cell growth and/or induced cell death in these cell lines. Treatment with α IR-3, an antibody blocking the binding domain of IGF-1R, also resulted in growth arrest and/or apoptosis. We also analyzed lymph node metastases of malignant melanoma by Western blotting and immunohistochem. All these cases were shown to express IGF-1R at the cell surface. In three cases of lymph node metastases we had access to both tumor specimens and cultured cells. One of these exhibited a substantially higher expression of IGF-1R than the two other cases. The corresponding cell lines showed growth arrest and apoptosis following treatment with α IR-3. However, the two cell lines with low expression of IGF-1R were more sensitive in this respect. Furthermore, we demonstrated an inverse correlation between IGF-1R expression and the frequency of apoptotic cells in the tumor specimens. Our data suggest that IGF-1R is crucial for the viability of malignant melanoma cells in vitro as well as in vivo.
IT Lymph node
(neoplasm, metastasis, melanoma metastasis to; IGF-I receptor expression and its anti-apoptotic effect in malignant melanoma)

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>>> IMAGES ARE AVAILABLE ONLINE AND FOR EMAIL-PRINTS <<<

=> s p75
L28 1765 P75

=> s neurotrophin receptor
1650 NEUROTROPHIN
983 NEUROTROPHINS
2076 NEUROTROPHIN
(NEUROTROPHIN OR NEUROTROPHINS)
89919 RECEPTOR
66783 RECEPTORS
99939 RECEPTOR
(RECEPTOR OR RECEPTORS)
L29 407 NEUROTROPHIN RECEPTOR
(NEUROTROPHIN(W) RECEPTOR)

=> s 128 and 129
L30 208 L28 AND L29

=> s cancer? or tumor? or neoplas?
88115 CANCER?
73158 TUMOR?
25581 NEOPLAS?
L31 109389 CANCER? OR TUMOR? OR NEOPLAS?

=> s antibod?
L32 97772 ANTIBOD?

=> s 132.and 131
L33 63932 L32 AND L31

=> s 129 and 133
L34 322 L29 AND L33

=> s immunoconjugate or (conjugat? or link? or coupl?)
1229 IMMUNOCONJUGATE
1863 IMMUNOCONJUGATES
2279 IMMUNOCONJUGATE

(IMMUNOCONJUGATE OR IMMUNOCONJUGATES)

84377 CONJUGAT?

336456 LINK?

374001 COUPL?

L35 568977 IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)

=> s 135 and 134

L36 316 L35 AND L34

=> s 136 not py>2001

641685 PY>2001

L37 105 L36 NOT PY>2001

=> s 136 not py>2000

740172 PY>2000

L38 79 L36 NOT PY>2000

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L38 ANSWER 1 OF 79 PCTFULL COPYRIGHT 2007 Univentio on STN

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DESIGNATED STATES

DETD BACKGROUND OF THE INVENTION

The tumor necrosis factor receptor (TNF-R) family members play key roles in the regulation of cell survival and death decisions (Baker and Reddy, . . .

transmembrane proteins, this family includes a soluble secreted protein, e.g. OPG (Emery et al., 1998, J of Biol Chem, 273:14363-14367), and a gpi-linked protein, DcR1 (Degli-Esposti, 1999, J of Leukocyte Biology, 65:535-542).

to a nucleic acid molecule encoding a TRADEcc polypeptide or TRADEp polypeptide or portion thereof. In another embodiment, the agent is an antibody that recognizes a TRADE family member polypeptide. In still another embodiment, the activity is selected from the group consisting of . . .

antisense to a nucleic acid molecule encoding a TRADE family polypeptide or portion thereof. In one embodiment, the agent is an antibody that recognizes a TRADE family polypeptide.

In one embodiment, the disorder is a proliferative disease or disorder selected from the group consisting of- inflammation and neoplasia. In one embodiment, the neoplasia is a carcinoma. In one embodiment, the neoplasia is present in lung or prostate tissue. In one embodiment, the neoplasia is an adenocarcinoma. In another aspect, the invention pertains to a method for treating a subject having a carcinoma or. . .

pathway refers to any one of the signalling pathways known in the art which involve activation or

deactivation of the transcription factor NF_κB, and which are at least partially mediated by the NF_κB factor (Karin, 1998, *Cancer J* from *Scientific American*, 4:92-99; Wallach et al, 1999, *Ann Rev of Immunology*, 17:331-367). Generally, such NF_κB signaling pathway are responsive. . .

any one of the signaling pathways known in the art which involve the Jun amino terminal kinase (JNK) 10 (Karin, 1998, *Cancer J* from *Scientific American*, 4:92-99; Wallach et al, 1999, *Ann Rev of Immunology*, 17:331-367). This kinase is generally responsive to a number of extracellular. . .

techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. An isolated TRADE nucleic acid molecule may, however, be linked to other nucleotide sequences that do not normally flank the TRADE sequences in genomic DNA (e.g., the TRADE nucleotide sequences may be linked to vector sequences). In certain preferred embodiments, an isolated nucleic acid molecule, such as a cDNA molecule, also may be free of. . .

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As used herein, the term neoplasia refers to a proliferative disease or disorder resulting from uncontrolled or aberrant cell division. The term neoplasia includes malignant and non-malignant disorders. As used herein, the term adenocarcinoma refers generally to cancers of glandular epithelial cells and carcinoma refers to malignant epithelial tumors.

herein, the term vector refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a plasmid, which refers to a circular double stranded DNA loop into which additional DNA segments may. . . replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as recombinant expression vectors or simply expression vectors. In general, expression vectors of utility in recombinant. . .

a location or locations in the genome that differs from that in which it occurs in nature or which is operatively linked to DNA to which it is not normally linked in nature (i.e., a gene that has been operatively linked to a heterologous promoter).

As used herein, the term antibody is intended to include

immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which binds (immunoreacts with) an antigen, such as Fab and F(ab')₂ fragments, single chain antibodies, intracellular antibodies, scFv, Fd, or other fragments. Preferably, antibodies of the invention bind specifically or substantially specifically to TRADE molecules (i.e., have little to no cross reactivity with non-TRADE molecules). The terms monoclonal antibodies and monoclonal antibody composition, as used herein, refer to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of an antigen, whereas the term polyclonal antibodies and polyclonal antibody composition refer to a population of antibody molecules that contain multiple species of antigen binding sites capable of interacting

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with a particular antigen. A monoclonal antibody compositions thus typically display a single binding affinity for a particular antigen with which it immunoreacts.

cells, e.g., excessive or unwanted proliferation of cells or deficient proliferation of cells. In one embodiment, TRADE associated disorders include such as neoplasia or inflammation. Examples of TRADE associated disorders include: disorders involving aberrant or unwanted proliferation of cells, e.g., inflammation, neoplasia, apoptosis, or necrosis. Preferably, the cells undergoing unwanted proliferation in a TRADE-associated disorder are epithelial cells, e.g., of the lung, liver, brain, intestine, or prostate. Further examples of TRADE associated disorders include carcinomas, adenocarcinomas, and other neoplasias. TRADE-associated disorders may also include disorders that have been linked generally to aberrant TNF receptor activity or function, including Crohn's Disease (Baert and Rutgeerts, 1999, Int J Colorectal Dis, 14:47-51) and.

II. Methods of Use
The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) methods of modulating proliferation of a cell, b).

TRADE protein or production of TRADE protein forms which have decreased or aberrant activity compared to TRADE wild type protein. Moreover, anti-TRADE antibodies can be used to detect and isolate TRADE proteins, regulate the bioavailability of TRADE proteins,

5 and modulate TRADE activity e.g., modulate. . . liver, the brain, the prostate, the lung, or the intestine. In one embodiment, the detection method is performed to determine whether a neoplastic condition exists, e.g., a carcinoma or an adenocarcinoma. In one embodiment of the invention, the subject methods are used (e.g., to modulate. . .

Exemplary inhibitory agents include antisense TRADE nucleic acid molecules (e.g., to inhibit translation of TRADE mRNA), intracellular anti- TRADE antibodies (e.g., to inhibit the activity of TRADE protein), and dominant negative mutants of the TRADE protein. Other inhibitory agents that can be. . .

For stimulatory or inhibitory agents that comprise nucleic acids (including recombinant expression vectors encoding TRADE protein, antisense RNA, intracellular antibodies or dominant negative inhibitors), the agents can be introduced into cells of the subject using methods known in the art for. . .

5 5,166,320). Binding of the DNA-ligand complex to the receptor facilitates uptake of the DNA by receptor-mediated endocytosis. A DNA-ligand complex linked to adenovirus capsids which naturally disrupt endosomes, thereby releasing material into the cytoplasm can be used to avoid degradation of the complex. . .

be detected by an appropriate assay, for example by immunological detection of a produced protein,

- 25 - such as with a specific antibody, or by a functional assay to detect a functional activity of the gene product.

apoptosis. In a preferred embodiment, TRADE is modulated to enhance apoptosis of a epithelial cell, such as to promote the apoptosis in cancer cells, e.g., in the lung, liver, brain, intestine or prostate.

as a nucleic acid or a protein, a naturally-occurring target molecule of a TRADE protein (e.g., a TRADE binding protein), a TRADE

antibody, a TRADE agonist or antagonist, a peptidomimetic of a TRADE agonist or antagonist, or other small molecule. In one embodiment, the agent inhibits one or more TRADE activities. Examples of such inhibitory agents include, e.g., antisense TRADE nucleic acid molecules, anti-TRADE antibodies, and TRADE inhibitors. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in. . .

will be desirable are in the treatment of TRADE-associated disorders, including disorders

involving aberrant or unwanted proliferation of cells, e.g., inflammation or cancer. Preferably, the I 0 cells undergoing unwanted proliferation are epithelial cells, e.g., of the lung or prostate.

Further examples of TRADE associated disorders include carcinomas, - adenocarcinomas, and other neoplasias. TRADE disorders may also include disorders that have been linked generally to aberrant TNF receptor activity or function, including Crohn's Disease (Baert and Rutgeerts, 1999, Int J Colorectal Dis, 14:47-51).

acid molecules, comprising at least a first nucleotide sequence encoding a full-length TRADE protein, polypeptide or peptide having a TRADE activity operatively linked to a second nucleotide sequence encoding a non- TRADE protein, polypeptide or peptide, can be prepared by standard recombinant 1 5 DNA.

be modified such that they specifically bind to 1 5 receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors.

polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using

linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B., 1996, *supra*)... . . . Res. 24 (17): 3357 For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. et al., 1989, Nucleic Acid Res. 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a TPNA segment and a YDNA segment (Finn P.J. et al., 1996, . . .

et al., 1988, Bio-Techniques 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization I 0 triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

B. Isolated TRADE Proteins, Fragments Thereof, and Anti-TRADE Antibodies

Isolated TRADE proteins, and biologically active portions thereof can also be

used as modulating agents, as well as polypeptide fragments suitable for use as immunogens to raise anti-TRADE antibodies. In one embodiment, native TRADE proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein. . . .

also provides TRADE chimeric or fusion proteins. As used herein, a TRADE chimeric protein or fusion protein comprises a TRADE polypeptide operatively linked to a non- TRADE polypeptide. An TRADE

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polypeptide refers to a polypeptide having an amino acid sequence corresponding to TRADE. . . .

Within the fusion protein, the term operatively linked is intended to indicate that the I O TRADE polypeptide and the non-TRADE polypeptide are fused in-frame to each other.

HA epitope tag). A TRADE encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the TRADE protein.

for the treatment of disorders, e.g., as soluble antagonists of the TRADE ligand. Disorders that would benefit from such treatment include, e.g. cancer or Alzheimer's disease. Such Fc fusion proteins can be used as soluble antagonists of the TRADE ligand. Moreover, the TRADE-fusion proteins of the invention can be used as immunogens to produce anti-TRADE antibodies in a subject.

a GST polypeptide). A TRADE-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the TRADE protein.

paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity), such as human TRADE, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of- -CH2NH-, -CH2S-, -CH2-CH2-, -CH=CH- (cis and trans), -COCH2-, -CH(OH)CH2-, and -CH2SO-, by methods known in. . . . (-C(OH)CH2-); and Hruby, V. J., 1982, Life Sci 31:189-199 (-CH2-S-); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is -CH2NH-. Such peptide mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced. . . .

antagonists of a TRADE/TRADE binding protein interaction. Peptides can be produced as modified peptides, with nonpeptide moieties attached by covalent

I 0 linkage to the N-terminus and/or C-terminus. In certain preferred embodiments, either the carboxy-terminus or the amino-terminus, or both, are chemically modified. The most. . . .

An isolated TRADE protein, or a portion or fragment thereof, can also be used as an immunogen to generate antibodies that bind TRADE using standard techniques for polyclonal and monoclonal antibody preparation. A full-length TRADE protein can be used or, alternatively, the invention provides antigenic peptide fragments of TRADE for use as immunogens. . . . The antigenic peptide of TRADE comprises at least 8 amino acid residues and encompasses an epitope of TRADE such that an antibody raised against the peptide forms a specific immune complex with TRADE. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least. . . .

or an amino acid sequence of another TRADE family polypeptide and encompasses an epitope of a TRADE polypeptide such that an antibody raised against the peptide forms an immune complex with a TRADE molecule. Preferred epitopes encompassed by the antigenic peptide are regions of TRADE that are located on 10 the surface of the protein, e.g., hydrophilic regions. In one embodiment, an antibody binds substantially specifically to a TRADE molecule. In another embodiment, an antibody binds specifically to a TRADE polypeptide.

A TRADE immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for. . . . or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic TRADE preparation induces a polyclonal anti- TRADE antibody response.

- 58 - Accordingly, another aspect of the invention pertains to the use of anti- TRADE family polypeptide antibodies. Such antibodies can be used as agonists and/or antagonists of TRADE family polypeptides. In a preferred embodiment antibodies specifically recognize TRADEa or P and not another TRADE family polypeptide.

Polyclonal anti-TRADE antibodies can be prepared as described above by immunizing a suitable subject with a TRADE immunogen. The anti-TRADE antibody titer in the

immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized a TRADE polypeptide. If desired, the antibody molecules directed against a TRADE polypeptide Ig G can be isolated from the mammal (e.g., from the blood) and further purified. . . . such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti- TRADE antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (Kohler and Milstein, 1975, *Nature* 256:495-497) (see also, . . . aL, 1980, *J Biol Chem* 255:4980-83; Yeh et al., 1976, *Prod. Naff Acad. Sci USA* 76:2927-31; and Yeh et al., 1982, *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al., 1983, *Immunol Today* 4:72), the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp.

77-96) or triorna techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner, 1981, *Yale J Biol. Med.*, . . . as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds specifically to a TRADE polypeptide.

Known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti- TRADE monoclonal antibody (see, e.g., G. Galfre et al., 1977, *Nature* 266:55052; Gefter et al.

Somatic Cell Genet., cited *supra*; Lerner, *Yale J Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinary skilled worker will appreciate that there are many variations of such methods which also would be. . . .

unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridorna culture supernatants for antibodies that bind a TRADE molecule, e.g., using a standard ELISA assay.

As an alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-TRADE antibody can be identified and isolated

by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with a TRADE to thereby isolate immunoglobulin library members that bind a TRADE polypeptide. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 2701- and the Stratagene Sur)Z4PTM Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example,

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Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International. . .

Barbas et al., 1991, Proc. Natl. Acad Sci USA 88:7978-7982; and McCafferty et al., 1990, Nature 348:552

Additionally, recombinant anti- TRADE antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Patent. . .

USA 84:214-218; Nishimura et al., 1987, Canc. Res. 47:999-1005; Wood et al., 1985, Nature 314:446-449; and Shaw et al., 1988, J Natl Cancer Inst. 80:1553-1559); Morrison, S. L., 1985, Science 229:1202-1207; Oi et al., 1986, BioTechniques 4:214; Winter U.S. Patent 5,225,539; Jones et al., 1986, Nature 321:552-525; Verhoeyan et al., 1988, Science 239:1534; and Beidler et al., 1988, J Immunol. 141:4053

In addition, humanized antibodies can be made according to standard protocols such as those disclosed in US patent 5,565,332. In another embodiment, antibody chains or specific binding pair members can be produced by recombination between vectors comprising nucleic acid molecules encoding a fusion of a. . .

An anti- TRADE antibody (e.g., monoclonal antibody) can be used to isolate a TRADE polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. Anti- TRADE antibodies can facilitate the purification of natural TRADE polypeptides from cells and of recombinantly produced TRADE polypeptides expressed in host cells. Moreover, an anti- TRADE antibody can be used to detect a TRADE protein (e.g., in a cellular lysate or cell supernatant). .

Detection may be
1 5 facilitated by coupling (ie., physically linking
) the antibody to a detectable substance.

Accordingly, in one embodiment, an anti- TRADE antibody of the invention is labeled with a detectable substance. Examples of detectable substances include various , enzymes, prosthetic groups, fluorescent materials, luminescent. . .

Accordingly, in one embodiment, anti-TRADE antibodies can be used, e.g., intracellularly to inhibit protein activity. The use of intracellular antibodies to inhibit protein function in a cell is known in the art (see e.g., Carlson, J. R., 1988, MoL Cell.

In one embodiment, a recombinant expression vector is prepared which encodes the antibody chains in a form such that, upon introduction of the vector into a cell, the antibody chains are expressed as a functional antibody in an intracellular compartment of the cell. For inhibition of TRADE activity according to the inhibitory methods of the invention, an intracellular antibody that specifically binds the TRADE protein is expressed in the cytoplasm of the cell. To pre-prepare an intracellular antibody expression vector, antibody light and heavy chain cDNAs encoding antibody chains specific for the target protein of interest, e.g., TRADE, are isolated, typically from a hybridoma that secretes a monoclonal antibody specific for the TRADE protein. Hybridomas secreting anti- TRADE monoclonal antibodies, or recombinant anti- TRADE monoclonal antibodies, can be prepared as described above. Once a monoclonal antibody specific for TRADE protein has been identified (e.g., either a hybridoma-derived monoclonal antibody or a recombinant antibody from a combinatorial library), DNAs encoding the light and heavy chains of the monoclonal antibody are isolated by standard molecular biology techniques. For hybridoma derived antibodies, light and heavy chain cDNAs can be obtained, for example, by PCR amplification or cDNA library screening. For recombinant antibodies, such as from a phage display library, cDNA encoding the light and heavy chains can be recovered from the display package (e.g., phage) isolated during the library screening process. Nucleotide sequences of antibody light and heavy chain genes from which PCR primers or cDNA library probes can be prepared are known in the art. For. . .

Once obtained, the antibody light and heavy chain sequences are cloned into a recombinant expression vector using standard methods. To allow for cytoplasmic expression of the light and heavy chains, the nucleotide sequences

encoding the hydrophobic leaders of the light and heavy chains are removed. An intracellular antibody expression vector can encode an intracellular antibody in one of several different forms. For example, in one embodiment, the vector encodes full-length antibody light and heavy chains such that a full-length antibody is expressed 1 0 intracellularly. In another embodiment, the vector encodes a full-length light chain but only the VH/CH1 region of the heavy chain such that a Fab fragment is expressed intracellularly. In the most preferred embodiment., the vector encodes a single chain antibody (scFv) wherein the variable regions of the light and heavy chains are linked by a flexible peptide linker (e.g., (Gly4SerB) and expressed as a single chain molecule. To 1 5 inhibit TRADE activity in a cell, the expression vector encoding the anti- TRADE intracellular antibody is introduced into -the cell by standard transfection methods, as discussed herein.

An antibody or antibody portion of the invention can be derivatized or linked to another functional molecule (e.g., a peptide or polypeptide). Accordingly, the antibodies and antibody portions of the invention are intended to include derivatized and otherwise modified forms of the anti-TRADE antibodies described herein, including, e.g.,

antibodies conjugated to other molecules (e.g., antibodies or polypeptides which bind to other cell markers). For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., to create a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the

antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody is produced by crosslinking two or more

antibodies (of the same type or of different types, e.g., to create bispecific antibodies).

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Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such

linkers are available from Pierce Chemical Company, Rockford, IL.

Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, 10 horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction. . . . agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody 15 may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

In one embodiment, anti-TRADE antibodies can be used to target cells expressing TRADE molecules. For example, an antibody can be used which recognizes a TRADE family molecules or which specifically recognizes a single TRADE family molecule and not another TRADE family molecule, e.g., an antibody which recognizes TRADEP. In one embodiment, such an antibody-toxin conjugate comprising the antibody and a toxin molecule can be used to deplete cells bearing a TRADE family or a specific TRADE molecule (e.g., by ablation). In a preferred embodiment, an anti-TRADE immunotoxin is used to target a tumor cell, e.g., *in vivo* or *ex vivo*. As used herein, the term toxin is meant to include molecules that are toxic.

A wide variety of toxins are known in the art and may be conjugated to the antibodies of the invention (see Hertler and Frankel, 1989, *J Clin Oncol* 7:1932-1942).

1994, *Nature Structural Biology* 1:59-64), as does the sarcin-A toxin, derived from the mold *Aspergillus giganteus* (Lacadena et al., 1999, *Proteins*, 37:474-484). Antibody-toxin

conjugates which include ricin-A and similar toxins have been described previously] in U.S. Patent Nos. 4,590,017, 4,906,469, 4,919,927, and 5,980,896, which are. . . .

diphtheria toxin (from *Corynebacterium diphtheriae*) and inhibit protein synthesis (Foley et al., 1995, *J Biol Chem*, 270:23218-23225) can also be used in the antibody-toxin conjugates of the invention.

Antibody-toxin conjugates which include

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diphtheria toxin or related toxins which ADP-ribosylate the EF-2 have been described

previously, e.g., in U.S. Patent Nos. . . .

62:361-363). Antibody-toxin conjugates which include maytansinoid have been described previously in U.S. Patent No. 5,208,020.

In one embodiment, a toxin for use in the antibody-toxin conjugates of the invention is an oligosaccharide. For example, the oligosaccharide calicheamicin is a bacterial product which was identified as one of a. . . .

repair

mechanisms (Chaudhry et al., 1999, Biochem Pharmacol, 57:531-538). Calicheamicin

is a preferred toxin moiety for use in connection with the invention. Antibody

calicheamicin conjugates have been described (Sievers et al., 1999, Blood, 93:3678-3684; Lode et al., 1998, Cancer Research, 58:2925-2928). Other synthetic cytotoxic

t

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compounds, such as CC- 1 065, have similar DNA-fragmenting mechanisms as calicheamicin and are also. . . .

Pharmacol, 52:447-453). Antibody-toxin conjugates, in which calicheamicin is covalently attached to an antibody through disulfide bonds, have been described previously in U.S. Patent Nos. 5,773,001 and 5,739,116.

Molecular conjugates which include aerolysin have been described previously in U.S.

There are numerous methods known in the art, for conjugating a toxin to an

antibody such that the activity of the toxin is appropriately delivered upon binding of the

1 5 antibody to a cell (Ghose and Blair, 1987, Crit Rev Ther Drug Carrier Syst, 3):263-3 59;

Hermentin and Seiler, 1988, Behring. . . . iWitt.] 82:197). For example', when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the

linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in Monoclonal Antibody -Toxin Conjugates: Aiming the Magic Bullet, Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference. The components may also be linked genetically (see, Chaudhary et al., 1989, Nature 339:394, which is herein incorporated by reference).

For example, in one embodiment, a covalent linkage can be formed between the

antibody and the toxin. In some cases, the existing cell-binding portion of a toxin must first be removed or altered to suppress its non-specific activity

(Hertler and Frankel, 1989, J Clin Oncol 7:1932-1942). The covalent linkage of antibody to toxin generally involves formation of a thioester or a disulfide bond. For example, conjugate compounds can be prepared by using N-succinimidyl 2(pyridyldithio)propionate, which can generate a disulfide linkage between an antibody and a toxin (Colombatti et al, 1983, J Immunology, 131:3091-3095). Numerous types of disulfide-bond containing

- 68 -
linkers are known which can successfully be employed to conjugate the toxin moiety with a polypeptide. In one embodiment, linkers that contain a disulfide bond that is sterically hindered are preferred, due to their greater stability in vivo, thus preventing release of the toxin moiety prior to binding at the site of action. Other methods forming covalent linkages between have been described in U.S. Patent Nos. 4,894,443, 5,208,021] 4,340,535, and EP 44167.

one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, operably linked is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system).

proteins can be utilized in TRADE activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for TRADE proteins, for example.

Publication No. WO 96/01313). Accordingly, in another embodiment, the invention provides a recombinant expression vector in which a TRADE DNA is operatively linked to an inducible eukaryotic promoter, thereby allowing for inducible expression of a TRADE protein in eukaryotic cells.

a
DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to TRADE mRNA. Regulatory sequences operatively

linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA.

be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a TRADE transgene to direct expression of a TRADE protein to particular

cells. Methods for generating transgenic animals via embryo manipulation. . . .

kinase assays involve expressing the deletion constructs in a host cell, isolating the expressed protein protein, immunoprecipitating the expressed protein with an antibody, and incubating the immune complex with 31p labeled ATP. This reaction is then run on SDS-PAGE and autoradiographed. This method is. . . .

blot analysis where the protein lysates are separated by SDS-PAGE, transferred to a membrane (i.e. nitrocellulose or nylon) and probed with an antibody against a protein of interest. This method of detection is well known in the art.

of a TRADE molecule can be accomplished, e.g., by direct binding. In a direct binding assay, the TRADE protein could be coupled with a radioisotope or enzymatic label such that binding of the TRADE protein to a TRADE target molecule can be determined by. . . .

are also available for use in the subject assay. For instance, either TRADE or its cognate binding protein can be immobilized utilizing conjugation of biotin and streptavidin. For instance, biotinylated TRADE molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with TRADE but which do not interfere with binding of upstream or downstream elements can be derivatized to the wells of the plate, and TRADE trapped in the wells by antibody conjugation. As above, preparations of a TRADE -binding protein and a test modulating agent are incubated in the TRADE - presenting wells. . . . be quantitated. Exemplary methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the TRADE binding element, or which are reactive with TRADE protein and compete with the binding element; as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the binding element, either intrinsic or extrinsic activity. In the instance of the latter, the enzyme can be chemically conjugated or provided as a fusion protein with the TRADE -BP. To illustrate, the TRADE -BP can be chemically cross-linked or genetically fused with horseradish peroxidase, and the amount of protein trapped in the complex can be assessed with a chromogenic substrate. . . .

For processes which rely on immunodetection for quantitating one of the proteins trapped in the complex, antibodies against the protein, such as anti- TRADE antibodies, can be used. Alternatively, the protein to be detected in the complex can be epitope tagged in the forra- of a fusion protein which includes, in addition to the TRADE sequence, a second protein for which antibodies are readily available (e.g. from commercial sources). For instance, the GST fusion proteins described above can also be

- 86 -

used for quantification of binding using antibodies against the GST moiety.. Other useful epitope tags include myc-epitopes (e.g., see Ellison et aL, 1991, J Biol Chem 266:21150- 2115 7) which. . .

of TRADE are known in the art (see discussions above). In one embodiment, within the expression vector the TRADE-coding sequences are operatively linked to regulatory sequences that allow for constitutive or inducible expression of TRADE in the indicator cell(s). Use of a recombinant expression vector. . . that enhance or inhibit the activity of TRADE. In an alternative embodiment, within the expression vector the TRADE coding sequences are operatively linked to regulatory sequences of the endogenous TRADE gene (i.e., the promoter regulatory region derived from the endogenous gene). Use of a recombinant expression vector in which TRADE expression

- 87 -

is controlled by. . .

transcription is altered by a modulation in TRADE expression or activity, e.g., the 5' flanking promoter and enhancer regions, are operatively linked to a marker 1 5 (such as luciferase) which encodes a gene product, that can be readily detected.

catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a target- responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., chloramphenicol acetyl transferase), or detecting a target-regulated cellular I O response, e.g., apoptosis.. . . a epithelial cell. The hallmark of apoptosis is degradation of DNA. Early in the process, this degradation occurs in intermucleosomal DNA linker regions. The 1 5 DNA cleavage may yield double-stranded and single-stranded DNA breaks. Apoptosis can be measured in cells using standard techniques.. . .

of a TRADE molecule can be accomplished, e.g., by direct binding. In a direct binding assay, the TRADE protein could be coupled with a radioisotope or enzymatic label such that binding of the TRADE protein to a TRADE target molecule

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techniques well known in the art (e.g., biotinylation kit, Pierce
Chemicals, Rockford,
IL), and immobilized in the wells of streptavidin-coated 96 well plates
(Pierce
Chemical). Alternatively, antibodies reactive with TRADE but
which do not interfere
with binding of upstream or downstream elements can be derivatized to
the wells of the
plate, and TRADE trapped in the wells by antibody
conjugation. As above, preparations
of a TRADE -binding.protein and a test modulating agent are incubated in
the TRADE -
presenting wells of the. . . methods for detecting such complexes, in
addition to those

- 95 -
described above for the GST-immobilized complexes, include
immunodetection of
complexes using antibodies reactive with the TRADE binding
element, or which are
reactive with TRADE protein and compete with the binding element; as
well as enzyme-
linked assays which rely on detecting an enzymatic activity
associated with the binding
element, either intrinsic or extrinsic activity. In the instance of the
latter, the enzyme can
be chemically conjugated or provided as a fusion protein with
the TRADE -BP. To
illustrate, the TRADE -BP can be chemically cross-linked or
genetically fused with
horseradish peroxidase, and the amount of protein trapped in the complex
can be
assessed with a chromogenic substrate. . .

For processes which rely on immunodetection for quantitating one of the
proteins
1 5 trapped in the complex, antibodies against the protein,
such as anti- TRADE antibodies
can be used. Alternatively, the protein to be detected in the complex
can be epitope
tagged in the form of a fusion protein which includes, in addition to
the TRADE
sequence, a second protein for which antibodies are readily
available (e.g. from
commercial sources). For instance, the GST fusion proteins described
above can also be
used for quantification of binding using antibodies against
the GST moiety. Other useful
epitope tags include myc-epitopes (e.g., see Ellison et al., 1991, J Biol
Chem 266:21150-
21157) which includes. . .

of TRADE are
known in the art (see discussions above). In one embodiment, within the
expression

vector the TRADE-coding sequences are operatively linked to regulatory sequences that allow for constitutive or inducible expression of TRADE in the indicator cell(s). Use of 1 5 a recombinant. . . . that enhance or inhibit the activity of TRADE. In an alternative embodiment, within the expression vector the TRADE coding sequences are operatively linked to regulatory sequences of the endogenous TRADE gene (i.e., the promoter regulatory region derived from the endogenous gene). Use of a recombinant. . . .

whose transcription is altered by a modulation in TRADE expression or activity, e.g., the 5' flanking promoter and enhancer regions, are operatively linked to a marker (such as luciferase) which encodes a gene product that can be readily detected.

catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., chloramphenicol acetyl transferase), or detecting a target-regulated cellular

response,, e.g., apoptosis. For example,. . . in a epithelial cell. The hallmark of apoptosis is degradation of DNA. Early in the process, this degradation occurs in internucleosomal DNA linker regions. The DNA cleavage may yield double-stranded and single-stranded DNA breaks. Apoptosis can be measured in cells using standard techniques. For example,. . .

of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing. . . .

model. For example, an agent identified as described herein (e.g., a TRADE modulating agent, an antisense TRADE nucleic acid molecule, a TRADE-specific antibody, or a TRADE -binding partner) can be used in an animal model to determine the efficacy, toxicity, or side I 0 effects. . . .

V. Other Uses and Methods of the Invention
The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) methods of treatment, e.g., up- or down-modulating proliferation. . . . protein or production of TRADE protein forius which have decreased or aberrant activity compared to TRADE wild type protein.

Moreover, the anti-TRADE antibodies of the invention can be used to detect and isolate TRADE proteins, regulate the bioavailability of TRADE proteins, and modulate a. . . .

of a TRADE molecule in a sample, e.g., portions or fragments of the cDNA sequences identified herein (and the corresponding complete-gene sequences), antibodies that recognize TRADE family polypeptides or specific TRADE polypeptides, can be used in numerous ways to detect TRADE nucleic acid or polypeptide. . . .

A preferred agent for detecting TRADE protein is an antibody capable of I 0 binding to TRADE protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')2) can be used. The term labeled, with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term biological sample. . . . for detection of TRADE mRNA include Northern hybridizations and in situ hybridizations. In vitro techniques for detection of TRADE protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitation and immunofluorescence. In vitro techniques for detection of TRADE genomic DNA include Southern hybridizations. Furthermore, in vivo techniques for detection of TRADE protein include introducing into a subject a labeled anti-TRADE antibody. -For-example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

may be performed, for example, by utilizing pre-I 0 packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a. . . .

TRADE molecules can also be linked, conjugated, or administered with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, TRADE can be coupled to any substance known in the art to promote penetration or

transport across the blood-brain barrier such as an antibody to the transferrin receptor, and administered by intravenous injection. (See for example, Friden et al., 1993, Science 259: 373-377 which is incorporated by reference). Furthermore, TRADE can be stably linked to a polymer such as polyethylene glycol to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties.

be in a composition which aids in delivery into the cytosol of a cell. For example, a TRADE molecule may be conjugated with a carrier moiety such as a liposome that is capable of delivering the peptide into the cytosol of a cell. Such. . .

that measurement of the level of TRADE in a cell or cells such as in a group of cells, tissue or neoplasia, like will provide useful information regarding apoptotic state of that cell or cells. In addition, it can also be desirable to. . .

can include a reagent for determining expression of TRADE (e.g., a nucleic acid probe(s) for detecting TRADE mRNA or one or more antibodies for detection of TRADE proteins), a I O control to which the results of the subject are compared, and instructions for. . .

Tris-buffered saline solution (TB S) with 10% (w/v) normal swine serum for one hour. For non-liver sections, primary mAb was detected with biotin-conjugated goat anti-mouse/rabbit IgG (Dako) in TBS for 30 minutes. Staining was detected with streptABC complex/horseradish peroxidase (Dako) diluted 1: 1 00 in. . .

of the four specimens. Focal immunoreactivity was also found in smooth muscle, and two cases showed weaker endothelial staining. The four prostate cancer specimens gave similar results, with strong, diffuse staining in the glandular epithelium. This signal was stronger, presumably as a result of the. . .

ducts (3+), and intense

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panacinar cytoplasmic staining of hepatocytes (3+). In the hepatocellular carcinoma specimen, there was intense staining of tumor cells with both mAbs.

unlike TRADE, p75 NGF1 expression has been reported to be lost in malignant specimens and it is not expressed in metastatic tumor lines derived from the prostate. The growth inhibition mediated by the CD40 ligand on tumor cells may have therapeutic value (Hirano et al., 1999, Blood, 93:2999-3007).

Example 3. Immunochemical analysis of TRADE
A panel of twenty murine monoclonal antibodies (mAb) specific
for the TRADE
extracellular domain were prepared for analyzing TRADE protein
expression.

and adapted to grow in ascites. An affinity column with
immobilized Protein A (Pierce, Rockford, IL) was used to purify
monoclonal antibody
from ascites fluids. Antibody class and subclass were tested
by using Mouse Hybridoma
Subtyping kit as per manufacturer's instructions (Boehringer Mannheim,
Indianapolis,
IN).

Cell lines were obtained from the American Type
Culture Collection (Rockville, MD). Cells were stained with anti-TRADE
or isotype
matched control monoclonal antibodies at 10 μ g/ml. Binding of
primary antibody was
detected with goat F(ab)anti-murine IgG conjugated to biotin,
followed by
streptavidin-phycoerythrin (Southern Biotechnology Associates,
Birmingham, AL).

a colonic adenocarcinoma, CaCo2. Specifically, the bottom panels show
the
results of treatment of a human astrocytoma cell line with both
antibodies in the
1 5 presence and absence of TRADE-Fc fusion protein. The dotted lines
represent the anti-
TRADEoc #8 and # 1 6 (bottom left and bottom right panels,
respectively). The solid lines
represent the control mIgG 1 and the antibody (either #8 or #
1 6) in the presence of
TRADE-Fc fusion protein. Specificity was confirmed by competing away the
FACS
staining. . . expression of TRADE in each of these cell lines was
also
confirmed by RT-PCR using TRADE specific primers. Two other prostate
tumor cell
lines, LNCaP.FGC and DU145, as well as other colon tumor
lines, HCT116 and HT-29
were negative for TRADE expression by flow cytometry with these inAbs.
Transiently
transfected COS cells were analyzed. . .

once activated, enters the
nucleus and activates transcription from several key genes involved in
cell survival and
proliferation checkpoints (Karin, 1998, Cancer Jfom
Scientific American, 4:92-99).

TRAF6 (Khursigara et al,
1999, J of Biol Chemistry, 274:2597-2600). The p75GF also stimulates
apoptosis, and a
novel zinc finger containing protein, NRIF (neurotrophin
receptor interacting protein)
mediates this signal (Casademunt et al., 1999, EMBO Journal, 18:605
0-606 1). NRIF
binds two motifs in the intracellular region. . .

of 50-60,000 Mr was noted, representing the TRADE-Fc monomer. In non-reducing conditions, a species of approximately 120,000 Mr was noted, illustrating the disulphide-linked dimer form of TRADE-Fc. This dimeric form is expected to be a potent, soluble antagonist of the TRADE ligand.

in vitro kinase assays. Specifically, cDNAs encoding Flag-tagged proteins (or vector control) were expressed in 293T cells and lysates immunoprecipitated using anti-Flag antibody or control antibody. The immune-

complexes were subjected to kinase assays using 12p labelled ATP and examined by SDS-PAGE. The gels were dried and analyzed. . .

CLMEN 12 The method of claim 2, wherein the agent is an antibody that recognizes a TRADE family member polypeptide

24 The method of claim 15 or 16, wherein the agent is an antibody that recognizes a TRADE family polypeptide.

claim 29 or 30, wherein the disorder is a proliferative disease or disorder selected from the group consisting of. inflammation and neoplasia.

32 The method of claim 3 1, wherein the neoplasia is a carcinoma.

I 0

33 The method of claim 3 1, wherein the neoplasia is present in lung or prostate tissue.

34 The method of claim 3 1, wherein the neoplasia is an adenocarcinoma

=> d his

(FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007)

FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007

L1 3555 S P75
L2 1651 S NEUROTROPHIN RECEPTOR
L3 806 S L1 AND L2
L4 775765 S CANCER? OR TUMOR? OR NEOPLAS?
L5 108 S L3 AND L4
L6 490570 S ANTIBOD?
L7 6 S L6 AND L5
L8 2288 S TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE
L9 2288 S TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)
L10 420 S L9 AND L4
L11 95 S L10 AND L6
L12 21 S TARGET? AND L11
L13 1486130 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L14 26 S L13 AND L11
L15 8 S L14 AND L12
L16 1 S L15 NOT PY>2002
L17 3 S L14 NOT PY>2001

L18 108 S INSULIN () GROWTH FACTOR RECEPTOR
L19 819 S (IGF-1R) OR (IGF1R) OR (IGF 1R)
L20 914 S L19 OR L18
L21 7 S TYPE 1 AND L18
L22 417 S L19 AND L4
L23 83 S L22 AND L6
L24 18 S L23 AND L13
L25 2 S L24 NOT PY>2001

FILE 'REGISTRY' ENTERED AT 10:27:37 ON 10 MAY 2007
L26 0 S 5C3/CN
L27 0 S MC192/CN

FILE 'PCTFULL' ENTERED AT 10:28:00 ON 10 MAY 2007
L28 1765 S P75
L29 407 S NEUROTROPHIN RECEPTOR
L30 208 S L28 AND L29
L31 109389 S CANCER? OR TUMOR? OR NEOPLAS?
L32 97772 S ANTIBOD?
L33 63932 S L32 AND L31
L34 322 S L29 AND L33
L35 568977 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L36 316 S L35 AND L34
L37 105 S L36 NOT PY>2001
L38 79 S L36 NOT PY>2000

=> s 129 or 128
L39 1964 L29 OR L28

=> s 139/clm or 139/ab
321 NEUROTROPHIN/CLM
23953 RECEPTOR/CLM
30 NEUROTROPHIN RECEPTOR/CLM
((NEUROTROPHIN(W) RECEPTOR) /CLM)
172 P75/CLM
62 NEUROTROPHIN/AB
22 NEUROTROPHINS/AB
78 NEUROTROPHIN/AB
((NEUROTROPHIN OR NEUROTROPHINS) /AB)
13885 RECEPTOR/AB
4554 RECEPTORS/AB
16605 RECEPTOR/AB
((RECEPTOR OR RECEPTORS) /AB)
13 NEUROTROPHIN RECEPTOR/AB
((NEUROTROPHIN(W) RECEPTOR) /AB)
33 P75/AB
L40 192 ((NEUROTROPHIN RECEPTOR/CLM) OR (P75/CLM)) OR ((NEUROTROPHIN
RECEPTOR/AB) OR (P75/AB))

=> s 140 and 133
L41 128 L40 AND L33

=> s 141 and 135
L42 127 L41 AND L35

=> s 142 not py>2000
740172 PY>2000
L43 42 L42 NOT PY>2000

=> d kwic

L43 ANSWER 1 OF 42 PCTFULL COPYRIGHT 2007 Univentio on STN
ABFR L'invention concerne une molecule d'un acide nucleique isole codant pour
un polypeptide

capable de lier un recepteur de p75?NTR et une version purifiee dudit polypeptide capable de lier un recepteur de p75?NTR. Cette invention se rapporte a un procede pour produire un polypeptide purifie capable de lier un recepteur de p75?NTR, ainsi qu'a un oligonucleotide antisens comportant une sequence d'acide nucleique capable de s'hybrider specifiquement a une molecule d'ARNm codant le polypeptide ci-decrit. Elle decrit un procede pour produire un polypeptide capable de lier un recepteur de p75?NTR a un vecteur approprie, ainsi qu'un procede pour provoquer l'apoptose, un procede pour determiner les effets physiologiques, un procede pour HGR74 sous la forme d'un vecteur approprie, une composition pharmaceutique comprenant un polypeptide purifie capable de lier un recepteur de p75?NTR et un excipient pharmaceutiquement acceptable et, enfin, un procede pour identifier un compose qui est un inhibiteur d'apoptose.

DETD

Figure 1E
Expression of endogenous NADE protein in SK-N-MC human neuroblastoma cells. SK-N-MC cell lysate treated with ALLN is immunoprecipitated by anti-NADE antibody, and subjected to immunoblotting by same antibody. Human NADE protein transiently expressed in 293T cells and untreated gels were used for controls. Heavy chain bands are resulted from antibodies using immunoprecipitation.

protein A wild type NADE, muNADE (Cys102Ser) , and muNADE (Cys121Ser) proteins transiently expressed in 293T cells were detected by immunoblotting with anti-NADE antibody. Transfection methods are described in material and methods. The cell lysate extracted from the 5293T cells transfected with parental vector was used.

NADE and p7 5NTR The cell lysates extracted from 293T cells co-transfected with Myc-tagged NADE and p7 5NTR were co-immunoprecipitated by anti-Myc antibody, and subjected to immunoblotting by anti-p75 NTR antibody. The 30 lysates from the cells transfected with each plasmid and a parental vector were used as controls. Transfection methods are described in.

Upper panel; Immunoprecipitates of anti-Myc antibody (IgG1) from each sample were subjected to immunoblotting analysis by anti p75 NTR antibody. Middle and lower panels indicated the NTR 5 expression level of p75 and NADE proteins by immunoblotting, respectively. The immunoprecipitate of anti-FLAG antibody (IgG1) was used as a control.

Figure 3E
35 Activation of Caspase-2 and 3 and degradation of PARP in co-transfected 293T cells. The cell extracts from 293T cells transfected by each cDNA as indicated were analyzed by immunoblotting with anti-Caspase-2, Caspase-3, and PARP antibody. The level of a-tubulin was measured as a control.

pM 2-mercaptoethanol (2-ME) for 5 min, subjected to a 12.5 % SDS-PAGE and analyzed by Western blot with the anti-NADE polyclonal antibody. Df Interaction of NADE and its point mutants with p75NTR. The

interaction of mutants with p75NTR was measured by using the GST fusion. . . .

described isolated nucleic acid

10 molecule encoding a polypeptide capable of binding a p75 NTR receptor, the isolated nucleic acid is operatively linked to a promoter of RNA transcription. In yet another embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule encodes. . . .

This invention provides a vector which comprises the isolated 5nucleic acid encoding a polypeptide capable of binding a p75 NTR receptor, operatively linked to a promoter of RNA transcription. In an embodiment of the invention, where in the vector which comprises the isolated nucleic acid encoding a polypeptide capable of binding a p75 NTF] receptor,, 10 operatively linked to a promoter of RNA transcription is a plasmid. In another embodiment the above described isolated nucleic acid molecule which is a. . . .

prototrophy to an auxotrophic host, biocide resistance or resistance to heavy metals such as copper. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, 10 or introduced into the same cell by cotransformation.

This invention provides a monoclonal antibody directed to an epitope of a polypeptide capable of binding a p75 NTR receptor.

5In an embodiment the above described monoclonal antibody, said monoclonal antibody is directed to a mouse, rat or human polypeptide capable of binding a p75 NTR receptor.

The term antibody includes, by way of example, both 10 naturally occurring and non-naturally occurring antibodies.

Specifically, the term antibody includes polyclonal and monoclonal antibodies, and fragments thereof. Furthermore, the term antibody includes chimeric antibodies, wholly synthetic antibodies, and fragments thereof. Optionally, an 15 antibody can be labeled with a detectable marker.

Detectable markers include, for example, radioactive or fluorescent markers.

This invention provides a polyclonal antibody directed to an 20 epitope of the purified protein having the amino sequence as set forth in Figure 1G-1 (SEQ ID NO: -) . in a further embodiment the above described monoclonal or polyclonal antibodies are directed to the polypeptide capable of binding a p75 NTR receptor, having the amino sequence as set forth in 25 Figure. . . .

Polyclonal antibodies may be produced by injecting a host animal such as rabbit, rat, goat, mouse or other animal with the immunogen of this. . . . polypeptide capable of binding a p75 NTR receptor. The sera are extracted from the host animal and are screened to obtain polyclonal antibodies which are specific to the immunogen. Methods of screening for polyclonal antibodies 35 are well known to those of ordinary skill in the art such as those disclosed in Harlow & Lane, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratories, Cold Spring Harbor,

NY: 1988) the contents of which are hereby incorporated by reference.

5The monoclonal antibodies may be produced by immunizing for example, mice with an immunogen. The mice are inoculated intra-peritoneally with an immunogenic amount of the. . .

In the practice of the subject invention any of the above-described antibodies may be labeled with a detectable marker.

15 In one embodiment, the labeled antibody is a purified labeled

antibody. As used in the subject invention, the term antibody includes, but is not limited to, both naturally occurring and non-naturally occurring antibodies.

Specifically, the term antibody includes polyclonal and 20 monoclonal antibodies, and binding fragments thereof.

Furthermore, the term antibody includes chimeric antibodies and wholly synthetic antibodies, and fragments thereof.

Furthermore, the term antibody includes chimeric antibodies

25 and wholly synthetic antibodies, and fragments thereof. A detectable moiety which functions as detectable labels are well known to those of ordinary skill in the art. . . . The secondary enzymatic or binding step may comprise the use of digoxigenin, alkaline phosphatase, horseradish peroxidase, 8-galactosidase, fluorescein or streptavidin/biotin. Methods of labeling antibodies are well 35 known in the art.

Determining whether the antibody forms such a complex may be accomplished according to methods well known to those skilled in the art. In the preferred embodiment,. . .

The antibody may be bound to an insoluble matrix such as that used in affinity chromatography. As used in the subject invention, isolating the cells which form a complex with the immobilized monoclonal antibody may be achieved by standard methods well known to those skilled in the art. For example, isolating may comprise affinity chromatography using immobilized antibody.

Alternatively, the antibody may be a free antibody.

In this

15 case, isolating may comprise cell sorting using free, labeled primary or secondary antibodies. Such cell sorting methods are standard and are well known to those skilled in the art.

The labeled antibody may be a polyclonal or monoclonal antibody. In one embodiment, the labeled antibody I .s a purified labeled antibody. The term antibody includes, by way of example, both naturally occurring and non-naturally occurring antibodies. Specifically, the term antibody includes polyclonal and monoclonal antibodies, and fragments thereof. Furthermore, the term antibody includes chimeric antibodies and wholly synthetic antibodies, and fragments thereof. The detectable marker may be, for example,

radioactive or fluorescent. Methods of labeling antibodies
30 are well known in the art.

of the above described transgenic nonhuman
15 mammal, the DNA encoding a polypeptide capable of binding a
p75 IT' receptor is operatively linked to tissue specific
regulatory elements.

An apoptosis inducing compound is defined as a compound which
may be, but not limited to, antibodies, inorganic compounds,
organic compounds, peptides, peptidomimetic compounds,
10 polypeptides or proteins, fragments or derivatives which
share some or all properties, e.g. fusion proteins, . . .

mammalian

homologs of the *Caenorhabditis elegans* death gene ced-3,
which are required for mammalian apoptosis. Increased levels
of caspase-2 and caspase-3 have been linked to apoptosis.

a DNA molecule. In an embodiment of the
above described transgenic nonhuman mammal, the DNA encoding
a human HGR74 protein is operatively linked to tissue
specific regulatory elements.

wherein the compound is bound to a
solid support. In an embodiment of the above described
method, wherein the compound comprises an antibody, an
5inorganic compound, an organic compound, a peptide, a
peptidomimetic compound, a polypeptide or a protein. In an
embodiment of the above described. . .

cytoplasmic protein may be bound to a solid
support. Also the compound may be bound to a solid support
and comprises an antibody, an inorganic compound, an organic
compound, a peptide, a peptidomimetic compound, a polypeptide
or a protein.

the compound is bound to a
2 Osolid support. In an embodiment of the above described
method, wherein the compound comprises an antibody, an
inorganic compound, an organic compound, a peptide, a
peptidomimetic compound, a polypeptide or a protein. In an
embodiment of the above described. . .

cytoplasmic protein may be bound to a solid
support. Also the compound may be bound to a solid support
and comprises an antibody, an inorganic compound, an organic
compound, a peptide, a peptidomimetic compound, a polypeptide
or a protein.

Experimental Details

Results and Discussions

The p75 IITR is the first-isolated neurotrophin receptor and the
member of TNFR (tumor necrosis factor receptor) family (7,
8). However, its functional role and signaling pathway has
remained largely unclear (9) . The existence of. . .

of the alternative
splicing form. The endogenous NADE protein was also
confirmed in human neuroblastoma cell line, SK-N-MC by
immunoprecipitation using the anti-NADE antibody (Fig. 1e).

NADE protein

5can be detected only in the presence of the ubiquitin

inhibitor such as ALLN, suggesting that NADE is modified by ubiquitin conjugating system leading to subsequent degradation by the proteasome. The molecular size of NADE is estimated to 22 kDa by the SDS-PAGE, and. . .

Antibodies. The polyclonal anti-NADE antibody was prepared by immunization of GST-mouse NADE fusion protein into the rabbit. The NADE specific antibody was affinity purified by antigen coupled Sepharose 4B. The polyclonal anti-rat p75 ITI was kindly gifted from Dr. M. V. Chao. The monoclonal anti-Myc antibody (9E10) was purchased from BIOMOL. The polyclonal anti-Caspase-3 antibody (H-277) was purchased from Santa Cruz Biotechnology. The polyclonal Caspase-2 antibody was kindly gifted from Dr. Lloyd A. Greene. HRP conjugated anti-rabbit IgG was purchased from Bio-Rad.

lysed in 0.5 ml of RIPA buffer. The supernatant of centrifuge (100,000 x g) was mixed with 1 Ag of polyclonal anti-NADE antibody coupled Sepharose 4B, and

incubated for 4 hours at 4 OC. After washing, the gels were boiled by 30 Al of SDS-PAGE sampling buffer and subjected to 12.5 % of SDS-PAGE. Immunoblotting was performed by polyclonal anti-NADE antibody (2 Ag/ml). In Fig. 1ff 10 Ag 35 of cell lysate extracted from each transfected 293T cells were used for the detection. . .

[35S] methionine labeled, and in vitro- translated NADE protein was mixed with 5 Al of GST-rat p75 NTRI CD fusion protein or GST-coupled GSH-Sepharose 4B (Pharmacia) in 100 Al of NETN buffer (20 mM Tris-HCl pH 8.01 100 mM NaCl, 1 mM EDTA, 0.2 %. . .

cells by were lysed in 1 ml of NETN buffer and centrifuged (100,000 Ag). The supernatants were immunoprecipitated by 2 Ag of anti-Myc antibody coupled Protein G Sepharose 4B (Pharmacia) for 2 hours at 4 'C. Following the 5 times washing, gels were subjected to 7.5 % SDS-PAGE, and Western blot analysis by rabbit polyclonal anti-p75 NTR antibody.

12, Smith, R. A. & Baglioni, C. The active form of tumor necrosis factor is a trimer. J. Biol. Chem. 262, 6951-6954 (1987).

normal development or in -response to a variety of stimuli, including DNA damage, growth factor deprivation, and abnormal expression of oncogenes or tumor suppressor genes (1-3). Apoptosis induced by these various reagents appears to be mediated by a common set of downstream elements that act as. . . as well as knockout and transgenic mice (5-7). However, the molecular mechanism of pro-apoptotic signaling involved in p75NTR is not well characterized. Recently, tumor necrosis factor receptor-associated factor (TRAF) family proteins, FAP-1, and zinc finger proteins have been reported to interact with p75NTR (ICD) (8-12) However, . . .

Reagents and Antibodies. Mouse nerve growth factor (NGF) was obtained from Sigma. TO-PRO-3 iodide was obtained from Molecular Probes. The anti-ot-NADE polyclonal antibody was

prepared as described previously (10).

In *vi tro* binding assay. In *vitro*-translated [³⁵Si] methionine-labeled proteins were generated by using the TNT-coupled reticulocyte lysate system (Promega). Binding assay was performed as described previously (22).

reducing condition, suggesting that NES is necessary for self-association of NADE and that the regulation of nuclear export of NADE may be linked to the association or dissociation of NADE monomer. Three of the key hydrophobic residues of NES, Leu 94, Leu 97 and Leu 99, . . .

10) . The expression of endogenous NADE was confirmed by anti- α (-NADE antibody in 293T, PC12 and nnr5 cells (data not shown). Introduction of NADE mutants, N (1-60), N (1-120) and N (C121S) with. . .

NADE NES motif is crucial for those functions. NES motif has been reported to require for self-association of p53 and may be linked to regulation of subcellular localization and p53 activity (30). NADE NES may be also important for the regulation of sublocalization recruitment to p75NTR. . .

8. The isolated nucleic acid of claim 1 operatively linked to a promoter of RNA transcription.

CLMEN. . . 11 The isolated nucleic acid molecule of claim 3, wherein the nucleic acid molecule encodes human or mouse polypeptide capable of binding p75 NTR receptor.

12 The isolated nucleic acid molecule of claim 11, wherein the nucleic acid molecule encodes a polypeptide capable of binding p75 NTR receptor set forth in Figure 1G-1 (SEQ ID NO: -) -

13 The isolated nucleic acid molecule of claim 3, wherein the nucleic acid molecule encodes a polypeptide capable of binding p75 NTR receptor.

14 The isolated nucleic acid molecule of claim 9 wherein the polypeptide capable of binding p75 NTR receptor is mouse, rat or human protein.

18 A method of producing a polypeptide capable of binding p75 NTR receptor which comprises growing the host cells of claim 17 under suitable conditions permitting production of the polypeptide.

An antisense oligonucleotide having a nucleic acid sequence capable of specifically hybridizing to an mRNA molecule encoding a polypeptide capable of binding p75 NTR receptor.

29 A purified polypeptide capable of binding p75 NTR receptor.

30 A purified polypeptide capable of binding p75 NTR receptor encoded by the isolated nucleic acid of claim 1.

31 A purified unique polypeptide fragment of the polypeptide capable of binding p75 NTR receptor of claim

3 0 .

the same amino acid

sequence as set forth in Figure 1G-1 (SEQ ID NO: -).

3 3 The polypeptide capable of binding p75 NTR receptor of claim 30 having the amino acid sequence as set forth in Figure 1G-1 (SEQ ID NO:

34 The polypeptide capable of binding p75 NTR receptor of claim 33 which is a vertebrate polypeptide capable of binding p75 NTR receptor.

37 The polypeptide of claim 35 which comprises an amino acid sequence of NCLRILMGEELSN.

3 8 .The vertebrate polypeptide capable of binding p75 NTR receptor of claim 34 which is a mouse, rat, or human polypeptide capable of binding p75 NTR receptor.

39 A monoclonal antibody directed to an epitope of a polypeptide capable of binding p75 NTR receptor of claim 35.

40 A monoclonal antibody of claim 33 directed to a mouse, rat or human polypeptide capable of binding p75 NTR receptor.

41 A polyclonal antibody directed to an epitope of the polypeptide capable of binding p75 NTR receptor of claim 32.

42 A polyclonal antibody of claim 41 directed to a mouse, rat or human polypeptide capable of binding p75 ITI receptor.

43 A method of inducing apoptosis in cells which comprises expressing a polypeptide capable of binding p75 NTR receptor in the cells.

47 The transgenic nonhuman mammal of claim 46, wherein the DNA encoding a polypeptide capable of binding p75 ITI receptor is operatively linked to tissue specific regulatory elements.

48 A method of determining physiological effects of expressing varying levels of a polypeptide capable of binding p75 ITI receptor in a transgenic nonhuman mammal which comprises producing a panel of transgenic non human mammal expressing a different amount of polypeptide capable of binding p75 NTR receptor.

49 A method of producing a polypeptide capable of binding p75 NTR receptor into a suitable vector which comprises.:

- (a) inserting a nucleic acid molecule encoding the polypeptide capable of binding p75 NTR receptor into a suitable vector;
- (b) introducing the resulting vector into a suitable host cell;
- (c) selecting the introduced host cell for the expression of the polypeptide capable of binding p75 NTR receptor;
- (d) culturing the selected cell to produce the polypeptide capable of binding p75 NTR receptor; and
- (e) recovering the polypeptide capable of binding p75 NTR receptor produced.

50 A method of inducing apoptosis of cells in a subject comprising administering to the subject a purified polypeptide capable of binding p75 NTR receptor in an amount effective to induce apoptosis.

53 A pharmaceutical composition comprising a purified polypeptide capable of binding p75 NTR receptor of either claim 32 or 33 and a pharmaceutically acceptable carrier.

54 A pharmaceutical composition comprising an effective amount of a purified Polypeptide capable of binding p75 IT' receptor of either claim 32 or 33 and a pharmaceutically acceptable carrier.

55 A method of identifying a compound capable of inhibiting binding between p75 NTR receptor and a polypeptide capable of binding p75 NTR receptor comprising:

- a) contacting the compound with the polypeptide capable of binding to p75 ITI receptor under conditions permitting the binding of the Polypeptide capable of binding to p75 IT' receptor and p7 SNTR receptor to form a complex;
- b) contacting the p75 receptor with the mixture from step a); and
- c) measuring the amount of the formed complexes or the unbound p75 ITI receptor or the unbound polypeptide or any combination thereof.

56 A method of identifying a compound capable of inhibiting binding between p75 NTR receptor and a polypeptide capable of binding p75 NTR receptor, where said binding forms a complex between p75 IT' receptor and a polypeptide capable of binding p75 NTR receptor, comprising:

- a) contacting the compound with the p75 ITR receptor under conditions permitting the binding of the polypeptide capable of binding to p75 NTR receptor and p75 NTR receptor to form a complex;
- b) contacting the p75 NTR receptor with the mixture from step a); and
- c) measuring the amount of the formed complexes or the unbound p75 NTR receptor or the unbound polypeptide or any combination thereof.

20 57. The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a neurotrophin associated cell death executor.

58 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a human HGR74 protein.

59 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a musnade3a sequence as defined on Figure 1H.

60 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a hunade3al sequence as defined on Figure 1H.

61 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 ITR receptor is a hunade3a2 sequence as defined on Figure 1H.

62 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a ratnad3a sequence as defined on Figure 1H.

63 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a ratnad3b sequence as defined on Figure 1H.

64 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a musnade3b sequence as defined on Figure 1H.

65 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a humnadel sequence as defined on Figure 1H.

66 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a ratnadel sequence as defined on Figure 1H.

67 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a musnadel sequence as defined on Figure 1H.

68 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 ITR receptor is a humnade2 sequence as defined on Figure 1H.

compound comprising:

a) contacting a subject with an appropriate amount of the compound; and
b) measuring the expression level of a polypeptide capable of binding p75 NTR receptor gene and p75 14TR gene in the subject, an increase of the expression levels of a polypeptide capable of binding p75 NTR receptor gene and p75 NTR gene indicating that the compound is an apoptosis inducing compound.

72 A method for identifying an apoptosis inducing compound comprising:

a) contacting a cell with an appropriate amount of the compound; and
b) measuring the expression level of a polypeptide capable of binding a p75 NTR receptor gene and p75 NTR gene in the cell, an increase of the expression levels of polypeptide capable of binding p75 NTR receptor gene and p75 NTR gene indicating that the compound is an apoptosis inducing compound.

73 A method for screening cDNA libraries of a polypeptide capable of binding p75 NTR receptor using a yeast two-hybrid system using a p75 NTR intracellular domain as a target.

76 The method of claim 73 where the p75 NTR intracellular domain target is mammalian.

35 77. The method of claim 76 where the mammalian p75 NTR intracellular domain target is a rat, mouse or human p75 ITI intracellular domain target.

and caspase-3 activity to
cleave poly (ADP-ribose) polymerase and fragment
nuclear DNA in a cell by co-expression of polypeptide
NTR NTI
capable of binding p75 receptor and p75

79 A method to inhibit NF-KB activation in a cell with
NTR NTR
polypeptide capable of binding p75 receptor and p75

80 A method to detect a neurodegenerative disease- in a
subject by detecting expression levels of a polypeptide
NT NTII
capable of binding p75 receptor and p75

84 The transgenic nonhuman mammal of claim 83 where the DNA
encoding a human HGR74 protein is operatively linked to
tissue specific regulatory elements.

compound

comprising:

(a) contacting a subject with an appropriate amount of
the compound; and
(b) measuring the expression level of human HGR74
protein gene and p75 NTR gene in the subj ect, an
increase of the expression levels of human HGR74
protein and p75 NTR gene indicating that the compound
is an apoptosis inducing compound.

compound

comprising:

(a) contacting a cell with an appropriate amount of
the compound; and
(b) measuring the expression level of human HGR74
protein gene and p75 NTR gene in the cell, an
increase of the expression levels of human HGR74
protein gene and p75 NTR gene indicating that the
compound is an apoptosis inducing compound.

95 A method for screening cDNA libraries human HGR74
protein using a yeast two-hybrid system using a p75 NTR
intracellular domain as a target.

98 The method of claim 95 where the p75 NTR intracellular
domain target is mammalian.

99 The method of claim 98 where the mammalian p7 SITR
intracellular domain target is a rat, mouse or human
p75 NTR intracellular domain target.

100. A method to induce caspase-2 and caspase-3 activity to
cleave poly (ADP-ribose) polymerase and fragment
nuclear DNA in a cell by co-expression of human HGR74
NTR

protein and p75.

101. A method to inhibit NF-KB activation in a cell with
NTR

human HGR74 protein and p75

102. A method to detect a neurodegenerative disease in a
subject by detecting expression levels of human HGR74
NTR

protein and p75

103. The method of claim 102 wherein the subject is a mammal.

104. The method of claim 103, wherein the mammal is. . . . method of
identifying a compound, which is an

apoptosis inhibitor, said compound is capable of inhibiting specific binding between polypeptide capable of binding p75 ITR receptor and p75 NTR receptor, so as to

prevent apoptosis which comprises:

(a) contacting the polypeptide capable of binding p75 IT' receptor with a plurality of compounds under conditions permitting binding between a is known compound previously shown to be able to displace a polypeptide capable of binding p75 NTR receptor and the p75 NTF' receptor and the bound p75 NTR receptor to form a complex; and
(b) detecting the displaced polypeptide capable of binding p75 11TR receptor or the complex formed in step (a), wherein the displacement indicates that the compound is capable of inhibiting specific binding between the polypeptide capable of binding p75 11TR receptor and the p75 NTR receptor.

106. The method of claim 105, wherein the inhibition of specific binding between the polypeptide capable of binding p75 NTR receptor and the p75 NTR receptor affects

the transcription activity of a reporter gene.

107. The method of claim 106, where in step (b) the displaced polypeptide capable of binding p75 NTR receptor or the complex is detected by comparing the transcription activity of a reporter gene before and after the contacting with the compound in step (a), where a change of the activity indicates that the specific binding between the polypeptide capable of binding p75 NTR

0 0

receptor and the p75 NTR receptor is inhibited and the polypeptide capable of binding p75 NTR receptor is displaced.

108. The method of claim 105, wherein the p75 NTR receptor is bound to asolid support.

109. The method of claim 105, wherein the compound is bound to a solid support.

110. The method of claim 105, wherein the compound comprises an antibody, an inorganic compound, an organic compound, a peptide, a peptidomimetic compound, a polypeptide or a protein.

111. The method of claim 105 wherein. . . . wherein the contacting or step

(a) is in a mammalian cell.

115. The method of claim 105, wherein the polypeptide capable of binding p75 NTR receptor is a cell surface receptor.

116. The method of claim 112, wherein the cell-surface receptor is the p75 receptor.

117. The method of claim 105 where in the polypeptide capable of binding p75 NTR receptor is a neurotrophin associated cell death executor.

118. The method of claim 105 where in the polypeptide capable of binding p75 IT' receptor is a human HGR74 protein.

119. The method of claim 105 wherein the polypeptide capable of binding p75 ITR receptor is a neurotrophin associated cell death executor.

120. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a human HGR74 protein.

121. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a musnade3a sequence as defined on Figure 1H.

122. The method of claim 105 wherein the polypeptide capable

of binding p75 NTR receptor is a hunade3al sequence as defined on Figure 1H.

123. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a hunade3a2 sequence as defined on Figure 1H.

124. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a ratnad3a sequence as defined on Figure 1H.

125. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a ratnad3b sequence as defined on Figure 1H.

126. The method of claim 105 wherein the polypeptide capable of binding p75 11TR receptor is a musnade3b sequence as defined on Figure 1H.

127. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a humnadel sequence as defined on Figure 1H.

128. The method of claim 105 wherein the polypeptide capable of binding p75 ITR receptor is a ratnadel sequence as defined on Figure 1H.

129. The method of claim 105 wherein the polypeptide capable of binding . . . SIT' receptor is a musnadel sequence as defined on Figure 1H.

130. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a humnade2 sequence as defined on Figure 1H.

131. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin is associated cell death executor protein (NADE), wherein the N-terminal 1-40 amino acids of wild type NADE polypeptide have. . . presence of p75NTR-

132. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin associated cell death executor protein (NADE), wherein the C-terminal 72-124 amino acids of wild type NADE polypeptide have been deleted and the deletion mutant is designated NADE N(1-71), and the NADE N(1-71) induces apoptosis in the presence of p75 NTR and in the absence of NTR

p75

133. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin associated cell death executor protein (NADE), wherein the N-terminal 1-40 amino acids and the C-terminal 72-124 amino acids. . . of p7 5NTR.

135. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin associated cell death executor protein (NADE), wherein the C-terminal 113-124 amino acids of wild type NADE polypeptide have been deleted and the deletion mutant is designated NADE N(1-112) and the NADE N(1-112) induces NTR

apoptosis in the presence of p75

136. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin associated cell death executor protein (NADE), wherein the C-terminal 101-124 amino acids of wild type NADE polypeptide have been deleted and the deletion mutant is designated NADE N(1-100) and the NADE N(1-100) induces

apoptosis in the presence of p75 NTR and in the absence of NTR

p75

137. An isolated nucleic acid molecule encoding a mutation of NTR

a wild type polypeptide capable of binding with a p75 receptor, designated neurotrophin associated cell death executor protein (NADE) , wherein the point mutation results in Ala at amino acid position 99 for. . . wild type NADE polypeptide, wherein the substitution mutant polypeptide is designated NADE N(L99A) and the NADE N(L99A) induces ITI

apoptosis in the presence of p75 .

Mouse NADE 1 MANVHQENEEMFQPLQNGEEDRPVGGGEGHQPAGNNNNNNHHNM

Human NADE 1 MANIHQENEEMEQPQMONGEEDRPLGGGEGHQPAGN -----

Mouse NADE 51 GQARRLAPNFRWAIPNRQMNNDGLGGDGGDMEMFMEEMREIER:K::L]RE

Human NADE 38 GQARRLAPNFRWAIPNRQINDGMGGDGGDMEIFMEEMREIRRKLRE

v

Mouse NADE 101 E]L]]]HDHHDEFCLMP 124

Human NADE. . . NYHD ---- FCLIP 97

humadel IFMEEMREIRRKLR EQLRNCLRI124GELS NHHDHHDEFCLMP

ratnadel MFMEEEMREIRRKLR EQLRNCLRIIMGELS NHHDHHDEFCLMP

musnadel EF7MEMREIRRKLR EQLRNCLRIIMELS NHHDHHDEFCLMP

hunmade2 RFMEEEMRELRRKIRE LQLRYSRLIIGDPP -HHDBHDEFCLMP

Figure 1H

/29

Figure 2A

kD

48

3 4

28

NADE

21

Figure 2B

4;]

Y.

N

kD

103

77 p75 NTR

48 mm]

344 .9*- rotein G

/29

NGF

(ng/ml)

0 10 100

IP: myc

4-p75NTR

WB:p75NTR

WB:p75NTR A=p75NTR

WB:myc .4w NADE monomer

Figure 2 C

/29

Control NADE

p75NTR NADE + p75NTR

Figure 3 A

/29

Figure. . . 8A

/29

GFP TO-PRO3

GFP-vector

GFP-NADE (WT.)

GFP-N (L99A)

GFP-N (L94A, L97A, L99A)
FIGURE 8B
/29
plcl]
q
]N
2-ME:] +] +] +
84,0
41.0 -o* Dimer
31,7
-- A* Monomer
1819
FIGURE 8C
/29
Oj
Se
OQS #4]
C,
*4w
Oj Oj
40
GST-p75 (ICD) + +
GST + + +
NOWN
FIGURE 8D
/29
40
30
20
CL
0
CL
lom
0
CP Jjcl
CN
FIGURE 8E
/29
M NGF
60- MNGF +
40-
20-
4)
T
0
p75NTR + + +
MNADE MIMI,
FIGURE 9A
FIGURE 9C
FIGURE 9B PC.

=> d his

(FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007)

FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007

L1 3555 S P75
L2 1651 S NEUROTROPHIN RECEPTOR
L3 806 S L1 AND L2
L4 775765 S CANCER? OR TUMOR? OR NEOPLAS?
L5 108 S L3 AND L4
L6 490570 S ANTIBOD?
L7 6 S L6 AND L5

L8 2288 S TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE
L9 2288 S TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)
L10 420 S L9 AND L4
L11 95 S L10 AND L6
L12 21 S TARGET? AND L11
L13 1486130 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L14 26 S L13 AND L11
L15 8 S L14 AND L12
L16 1 S L15 NOT PY>2002
L17 3 S L14 NOT PY>2001
L18 108 S INSULIN () GROWTH FACTOR RECEPTOR
L19 819 S (IGF-1R) OR (IGF1R) OR (IGF 1R)
L20 914 S L19 OR L18
L21 7 S TYPE 1 AND L18
L22 417 S L19 AND L4
L23 83 S L22 AND L6
L24 18 S L23 AND L13
L25 2 S L24 NOT PY>2001

FILE 'REGISTRY' ENTERED AT 10:27:37 ON 10 MAY 2007

L26 0 S 5C3/CN
L27 0 S MC192/CN

FILE 'PCTFULL' ENTERED AT 10:28:00 ON 10 MAY 2007

L28 1765 S P75
L29 407 S NEUROTROPHIN RECEPTOR
L30 208 S L28 AND L29
L31 109389 S CANCER? OR TUMOR? OR NEOPLAS?
L32 97772 S ANTIBOD?
L33 63932 S L32 AND L31
L34 322 S L29 AND L33
L35 568977 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L36 316 S L35 AND L34
L37 105 S L36 NOT PY>2001
L38 79 S L36 NOT PY>2000
L39 1964 S L29 OR L28
L40 192 S L39/CLM OR L39/AB
L41 128 S L40 AND L33
L42 127 S L41 AND L35
L43 42 S L42 NOT PY>2000

=> s 143 and dox? or (paclitaxel or taxol or taxane)

19784 DOX?
8111 PACLITAXEL
66 PACLITAXELS
8113 PACLITAXEL
(PACLITAXEL OR PACLITAXELS)
8165 TAXOL
240 TAXOLS
8235 TAXOL
(TAXOL OR TAXOLS)
1713 TAXANE
2293 TAXANES
3137 TAXANE
(TAXANE OR TAXANES)

L44 13134 L43 AND DOX? OR (PACLITAXEL OR TAXOL OR TAXANE)

=> s 143 and (dox? or (paclitaxel or taxol or taxane))

19784 DOX?
8111 PACLITAXEL
66 PACLITAXELS
8113 PACLITAXEL
(PACLITAXEL OR PACLITAXELS)
8165 TAXOL

240 TAXOLS

8235 TAXOL

(TAXOL OR TAXOLS)

1713 TAXANE

2293 TAXANES

3137 TAXANE

(TAXANE OR TAXANES)

L45

3 L43 AND (DOX? OR (PACLITAXEL OR TAXOL OR TAXANE))

=> d ibib 1-3

L45 ANSWER 1 OF 3

PCTFULL COPYRIGHT 2007 Univentio on STN

ACCESSION NUMBER:

2000073321 PCTFULL ED 20020515

TITLE (ENGLISH):

HUMAN TUMOR NECROSIS FACTOR RECEPTOR TR10

TITLE (FRENCH):

TR10, RECEPTEUR DE FACTEUR DE NECROSE TUMORALE

HUMAIN

INVENTOR(S):

ROSEN, Craig, A.;

NI, JianRP : KLEIN, Jonathan, L.

PATENT ASSIGNEE(S):

HUMAN GENOME SCIENCES, INC.;

ROSEN, Craig, A.;

NI, Jian

English

Patent

LANGUAGE OF PUBL.:

DOCUMENT TYPE:

PATENT INFORMATION:

| NUMBER | KIND | DATE |
|--------|------|------|
|--------|------|------|

WO 2000073321 A1 20001207

DESIGNATED STATES

W:

AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE
DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE
KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX
NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA
UG US UZ VN YU ZA ZW GH GM KE LS MW MZ SD SL SZ TZ UG
ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI
FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN
GW ML MR NE SN TD TG

PRIORITY INFO.:

US 1999-60/136,786 19990528

US 1999-60/142,563 19990707

US 1999-60/144,023 19990715

APPLICATION INFO.:

WO 2000-US14554 A 20000526

L45 ANSWER 2 OF 3

PCTFULL COPYRIGHT 2007 Univentio on STN

ACCESSION NUMBER:

1999045944 PCTFULL ED 20020515

TITLE (ENGLISH):

PROAPOPTOTIC PEPTIDES, DEPENDENCE POLYPEPTIDES AND

METHODS OF USE

TITLE (FRENCH):

PEPTIDES PROAPOPTOTIQUES, POLYPEPTIDES DE DEPENDANCE,

ET MODE D'UTILISATION

INVENTOR(S):

BREDESEN, Dale, E.;

RABIZADEH, Shahrooz

THE BURNHAM INSTITUTE

English

Patent

PATENT ASSIGNEE(S):

LANGUAGE OF PUBL.:

DOCUMENT TYPE:

PATENT INFORMATION:

| NUMBER | KIND | DATE |
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WO 9945944 A1 19990916

DESIGNATED STATES

W:

AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC

NL PT SE

PRIORITY INFO.:

US 1998-09/041,886 19980312

APPLICATION INFO.:

WO 1999-US5250 A 19990311

L45 ANSWER 3 OF 3

PCTFULL COPYRIGHT 2007 Univentio on STN

ACCESSION NUMBER:

1994022866 PCTFULL ED 20020513

TITLE (ENGLISH): PYRAZOLOQUINAZOLONE DERIVATIVES AS NEUROTROPHIC AGENTS
 TITLE (FRENCH): DERIVES DE LA PYRAZOLOQUINAZOLONE EN TANT QU'AGENTS
 NEUROTROPES
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 CAPRATHE, Bradley, William
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L45 ANSWER 2 OF 3 PCTFULL COPYRIGHT 2007 Univentio on STN

DETD . . . factors, extracellular matrix, CD40 ligand, viral gene products, zinc, estrogen and androgens. In contrast, stimuli which promote apoptosis include growth factors such as tumor necrosis factor (TNF), Fas, and transforming growth factor] (TGF]), growth factor withdrawal, loss of extracellular matrix attachment, intracellular calcium and glucocorticoids, for example. . . .
 cellular apoptosis. Thus, the peptides are useful for the treatment of various pathological conditions characterized by unregulated cell growth or survival such as cancer, autoimmune and fibrotic disorders. Moreover, proapoptotic dependence peptides derived from negative signaling polypeptides are advantageous in that they can be used for the identification. . . .
 be performed using methods well known to those skilled in the art. Such methods include, for example, affinity and immunoaffinity selection using ligands, antibodies and anti-idiotype antibodies, for example.
 known to those skilled in the art. For example, changes in conformation can be assessed by, for example, determining the binding of conformation-specific antibodies or other binding probes, construction and testing of methods known or predicted to influence conformational changes or stability of a polypeptide or by. . . .
 nonspecific endocytosis, or through the use of heterologous targeting domain. For example, in a particular embodiment described below, an HIV tat protein, when linked to a dependence peptide, facilitates cellular entry. Lipid carriers also can be used to introduce the nucleic acids encoding proapoptotic dependence peptides, or. . . .
 such a heterologous

functional domain that facilitates entry into a cell is the HIV tat protein. This protein or functional equivalents thereof, when coupled to a proapoptotic dependence peptide increases the apoptotic activity of the peptide 30-fold compared to the peptide alone.

to those skilled in the art. Such domains include, for example, ligands to extracellular proteins or receptors, ligands to other cell surface receptors, antibodies, a natural or engineered viral protein with a 30 desired cell tropism, toxin subunits which facilitate toxin entry and functional fragments thereof.

A heterologous functional domain also can augment the cell death activity of the proapoptotic dependence peptide by linking one or more additional cell death or inhibitory activities onto the proapoptotic dependence peptide. Such cell death or inhibitory activities include, for example, . . . activity. Domains which exhibit apoptotic activity include, for example, ligands or agonists to receptors which induce programmed cell death. Fas ligands or anti-Fas antibodies are two specific examples of such apoptotic domains. A domain which activates caspase protease activity is another example of a heterologous functional domain which exhibits apoptotic activity. Domains which exhibit cytotoxic or cytostatic activity include, for example, toxins and chemotherapeutic agents such as doxorubicin, methotrexate, vincristine and cyclophosphamide can be conjugated to a dependence peptide. Other agents exist as well and are known to those skilled in the art and can be linked to proapoptotic peptides to augment their cell death function.

A heterologous functional domain also can be a regulatable moiety that modulates the activity of a proapoptotic dependence peptide. When linked to a proapoptotic dependence peptide, a modular domain can impart ligand dependent activation or repression of its proapoptotic activity. For example, many different ligand-dependent. . .

if hydrophobic, can directly enter cells. Alternatively, in the event that the dependence polypeptide or functional equivalent is intracellular, a test compound can be

P conjugated to a targeting moiety, for example, the HIV tat protein, to facilitate cell entry. Incorporation of the test compound into liposomes is. . .

example, the loss of apoptosis in cells can lead to the pathological accumulation of self-reactive lymphocytes, virally infected cells, hyperproliferative cells such as neoplastic or tumor cells and cells that contribute to fibrotic conditions. Inappropriate activation of apoptosis also can contribute to a variety of pathological disease states including, . . .

survival consisting of cytoplasmically administering a proapoptotic dependence peptide. Further provided is a method of reducing the severity of a pathological

condition consisting of neoplastic, malignant, autoimmune or fibrotic conditions by cytoplasmically administering a proapoptotic dependence peptide.

resistance to serum proteases additionally can be used as well as other formulations known in the art. For the treatment of a neoplastic or fibrotic condition, the proapoptotic dependence peptide can be administered by direct injection into a solid tumor mass or into a region of fibrosis. Additional modes of administration are known and can be determined by those skilled in the. . .

vectors containing a natural or engineered envelope protein also can be used to target a nucleic acid encoding a proapoptotic dependence peptide to neoplastic, malignant or autoimmune tissues of cells expressing an appropriate cell surface protein. Thus, disorders characterized by cells that abnormally proliferate can be selectively. . .

The nucleotide sequence of all constructs was confirmed by DNA sequencing. The expression of p75TR protein was detected by flow cytometry using monoclonal antibody 192, and immunoblotting using anti-p75 antiserum (Promega, Madison, WI).

Acad. Sci. USA 90:4533-4537 (1993)). The expression of p75 ITI protein in the transfected cells was detected by flow cytometry using the monoclonal antibody 192 (Baldwin et al. J.

FKBP/p75 NTP% protein chimeras containing one or three copies of an FKBP fused to an intracytoplasmic form of p75NTI were expressed in cells. Cross-linking studies indicated that FKBP expressed in cells could be induced to form dimers or multimers by exposing the cells to the FK1012. . .

of some of the peptides tested. This HIV tat sequence did not affect the function of the peptide to which it was linked, as shown below. For convenience, the hyphen in the above amino acid sequences is a nomenclature intended to set apart the proapoptotic dependence. . .

Peptides which did not exhibit apoptotic activity without the amino-terminal tat sequence similarly did not exhibit apoptotic activity with the linked tat sequence. Specifically, cell viability after exposure to tat-purple was 97.8% for COS-7, 92.8% for PC3 and 69.3% for Ntera 2 cells.. . .

To further analyze the effect of particular point mutations on apoptosis, additional studies employing dependence peptides and mutated variants linked to tat were performed in SH-SY5Y cells. The results shown in Figure 2 are of studies in which quadruplicate samples were averaged, and. . .

and peptide was added to the mitochondria at a final concentration of 385 uM. Western blot analysis using a cytochrome c specific antibody

monitored the amount of cytochrome c released (Ellerby et al. J. Neurosci. - 17:6165-6178 (1997)).

were washed and resuspended in CFS (50-100 mg/ml) and the final peptide concentration was 385 μ M. Western blot analyses using the caspase-3 specific antibody, CPP32, was performed as described (Ellerby et al. J. Neurosci. 17:6165-6178 (1997)).

CLMEN. . . pure proapoptotic dependence peptide comprising substantially the sequence of an active dependence domain selected from the group of 5 dependence polypeptides consisting of p75', androgen receptor, DCC, huntingtin polypeptide, Machado-Joseph disease gene product, SCA1, SCA2, SCA6 and atrophin-1 polypeptide.

2 The proapoptotic dependence peptide of claim 1, wherein the dependence polypeptide is p75 IT' and the proapoptotic dependence peptide further comprises substantially the sequence selected from the group consisting of SATLDALLAALRRI (SEQ ID NO:3), SATLDALLAALGGI (SEQ ID. . .

19 The method of claim 18, wherein said proapoptotic dependence domain-mediated apoptosis is induced by unliganded p75'.

29 The method of claim 28, wherein said pathological condition comprises neoplastic, malignant, autoimmune or fibrotic conditions.

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L45 ANSWER 3 OF 3 PCTFULL COPYRIGHT 2007 Univentio on STN
ABEN . . . salts thereof, methods of production, intermediates in their production, pharmaceutical compositions containing said compounds, and methods for treating neurodegenerative disorders, tumors of neuronal origin, inflammation, allergy, and pain, and methods for screening compounds that interact with the neurotrophic receptors using said. . .

DETD . . . ART
Nerve growth factor (NGF) was first described by Levi-Montalcini (j, Exp, Zool., 116:321-362 (1951)) as an activity secreted by a mouse sarcoma tumor implanted into a chick embryo. Both sensory ganglion and sympathetic ganglion neurons grew neurites into the sarcoma, which also supported the growth of. . .

(Kaplan, et al., Science, 252:554-558 (1991)). More specifically, NGF prevents the development of small-fiber sensory neuropathies that result from the use of taxol, a chemotherapeutic agent (Apfel, et al., Ann. Neurol., 29:87-90 (1991)). NGF is also efficacious against the development of large-fiber sensory neuropathies resulting from the. . .

Effects of NGF on Neuronal Tumors
The importance of NGF in the formation of neuronal tumors has not been firmly established. Certain

investigators believe that excessive synthesis of peptide growth factors or their receptors may lead to the transformation of the recipient cells (Levi-Montalcini, *Science*, 237:1154-1162 (1987)). In fact, it has been shown that brain tumors secrete a variety of growth factors, including NGF (Westermann, et al., *J. Neurochem.*, 50:1747-1758 (1988)), BDNF (Lichtor, et al., *Mol. Cell Neurosci.*, 2:168-171 (1991)). . .

Res. Pract., 185:332-338 (1989)), This suggests that agents that block the effects of NGF may be beneficial in the treatment of neuronal tumors, On the other hand, NGF has been utilized as a reverse transforming agent to halt the progression of animal tumors of neurodegeneric origin (Yaeger, et al., *Acta Neuropathol.*, 83:624-629 (1992)).

exists evidence to suggest that both blockade and stimulation of the effects of NGF may be beneficial for controlling the abnormal growth of tumors of neuronal origin. Any type of agent that modulates the interaction between NGF and its receptors may be considered as a potential. . .

dependent on NGF for survival, both in vitro and in vivo. For example, neutralization of NGF activity in newborn animals by administration of antibodies against NGF interferes with the normal development of sensory and sympathetic neurons (Rohrer, et al., *Development*, 103:545-552 (1988)), An important recent finding is. . . innervating the point of blockade, and may be a viable option for pain control in certain patients, such as those with terminal stage cancer or those undergoing limb removal.

through molecular biology techniques to express exclusively 1 receptor or the other, these cell lines (e.g., PC12 or NIH-M) are usually derived from tumors and is receptor expression levels tend to be much higher than in native neurons. A method that would allow the study of the. . .

using 1,11-carbonyldiimidazole (8) as a coupling agent.

the PNS, such as drug-induced peripheral neuropathies and the like. The compounds are also useful as active agents in the treatment of tumors of neuronal origin, in the treatment of allergy and inflammation, as analgesic agents, as tools in the screening for agents with neurotrophic and antineurotrophic. . .

CLMEN 32 A method for treating tumors of neuronal origin in mammals which comprises administering an antitumor effective amount of a compound of the formula
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N']N
R1
N
1
X2
or a salt thereof, wherein

SUBSTITUTE. . .

compound being screened with a predetermined amount of radiolabeled neurotrophic factor in the presence of a predetermined amount of cells that express both p75 and a trk receptor and a predetermined amount of a compound of the formula
0
ANvoN
(al R1
N
h2
or a salt thereof, in a pharmaceutical. . .

32 A method for treating tumors of neuronal origin in mammals which comprises administering an antitumor effective amount of a compound of the formula
0
NopoN
R1
N
I
A2
or a salt thereof, wherein
AMENDED. . .

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L45 ANSWER 1 OF 3 PCTFULL COPYRIGHT 2007 Univentio on STN
TIEN HUMAN TUMOR NECROSIS FACTOR RECEPTOR TR10
TIFR RECEPTEUR DE FACTEUR DE NECROSE TUMORALE HUMAIN
ABEN The present invention relates to a novel protein, TR10, which is a member of the tumor necrosis factor (TNF) receptor superfamily and the TRAIL receptor subfamily. In particular, isolated nucleic acid molecules are provided encoding the. . .
ABFR . . . invention concerne une nouvelle proteine, la TR10, qui constitue un membre de la superfamille des recepteurs de facteur de necrose tumorale (TNF) et de la sous-famille de recepteur TRAIL. En particulier, cette invention concerne des molecules d'acide nucleique isolees codant. . .

DETD HUMAN TUMOR NECROSIS FACTOR RECEPTOR TR10
FIELD OF THE INVENTION
The present invention relates to a novel member of the tumor necrosis factor family of receptors. More specifically, isolated nucleic acid molecules are provided encoding a novel human tumor necrosis factor receptor, TRIO. TRIO polypeptides are also provided, as are vectors, host cells, and recombinant methods for producing the same, and antibodies that bind to TRIO polypeptides. The invention further relates to screening methods for identifying
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agonists and antagonists of TRIO activity.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines, which act through TNF receptors to regulate numerous biological processes, including protection against infection. . .

(R. Watanabe-Fukunaga et al., *Nature* 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation. . .

number of biological effects elicited by TNF and LT-, acting through their

LI In Z_ Z_ receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT- are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral

1_] Zn tn malaria, tumors, autoimmune disease, AIDS and graft-host rejection (B. Beutler and C. Von Huffel, *Science* 264:667-668 (1994)). Mutations in the p55 receptor cause. . .

and homeostasis of multicellular organisms (H. Steller, *Science* 267:1445-1449 (1995)). Derangements of apoptosis contribute to the pathogenesis of several human diseases including cancer, neurodegenerative disorders, and acquired immune deficiency syndrome (C.B. Thompson, *Science* 267:1456-1462 (1995)). Recently, much attention has focused on the signal transduction and. . .

detecting over-expression of TRIO, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors.

Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses. . .

dysregulation can lead to a number of different pathogenic processes. Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers, autoimmune disorders, viral infections, inflammation, graft vs. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include. . .

following tissues: fetal liver, peripheral blood lymphocytes (PBL), lung, kidney, small intestine, colon, endothelial cells, and monocyte activated tissue. Furthermore, the following cancer cell lines express TRIO: HeLa cell S3, SW480 (colorectal adenocarcinoma), and A549 (lung carcinoma).

prostate, testis, ovary, small intestine, colon, PBLs, lymph node, bone marrow and fetal liver. TRIO expression was not observed in most cancer cell lines tested. See Example 7, below.

Zn LI
(e.g., inhibit) hematopoiesis), anti crenicity (ability to bind (or compete with a TRIO polypeptide for binding) to an anti-TRIO antibody), immunogenicity (ability to generate antibody which binds to a TRIO polypeptide), ability to form multimers with TRIO polypeptides of the invention, and ability to bind to a. . .

one embodiment where one is assaying for the ability to bind or compete with full-length TRIO polypeptide for binding to anti-TRIO antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), sandwich immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels',. . . C, C] C] LI ' I I complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is -i detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting and binding in an immunoassay and are within the scope of. . .

a disease which results from under-expression over-expression or altered expression of TRIO or a soluble form thereof, such as, for example, tumors or autoimmune disease.

of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the In host, supplied by a complementary vector or supplied. . .

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp and tac promoters, the SV40. . .

applications, particularly those that make use of the chemical and biological properties of TRIO. Among these are applications in treatment of tumors, resistance to parasites, bacteria and viruses, to induce proliferation

of T-cells,' endothelial cells and certain hematopoietic cells, to treat restenosis araft vs. . . .
the animal, in situ hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transcrenic gene-expressing tissue may also be evaluated immunocytochemically or Immunohistochemically usinCF in antibodies specific for the transgene product.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation.

In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are fon-ned when proteins of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterolocrous polypeptide sequence in

t) zn a fusion protein of the invention) in solution. In other embodiments, multimers of the invention. . . recited in SEQ ID

In 2o NO:2 or the polypeptide encoded by the deposited cDNA clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another. . . are herein incorporated by reference in its entirety). In another embodiment, two or more TRIO polypeptides of the invention are through synthetic linkers (e.cr., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple TRIO polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

(each of which is hereby incorporated by reference). In further preferred embodiments, a TRIO-FLAG fusion protein is detectable by anti-FLAG monoclonal antibodies (also available from Slorma).

chemical techniques known in the art. For example, proteins desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.cy., US Patent Number 5,478,925, which is herein incorporated by reference in its. . . entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the polypeptide sequence of the proteins desired to

be contained in the multimer (see, e.cr., US. . . .

the invention are generated by ligating a polynucleotide sequence encoding a

C] In polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original. . . .

to bind TRIO

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licrand) may still be retained. For example, the ability of shortened TRIO mureins to induce

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and/or bind to antibodies which recognize the complete or mature forms of the polypeptides

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generally will be retained when less than the majority of the. . . .

and/or endothelial cells) may still be retained. For example the ability of the shortened TRIO murein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues. . . .

cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for

Ln t_1

example, expression of a hornocreneous product that is more easily recovered and purified from yeast hosts which are known to hypererlycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid. . . .

which are differentially modified during or after translation, e.cr., by glycosylation, acetylation, phosphorylation,

Z.- Zn

amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to

L in tn in

'body molecule or other cellular licrand, etc. Any of numerous chemical modifications

an anti t]

may be carried out. . . .

Additional post-translational modifications encompassed by the invention include, for example, e.cr., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptides may. . . .

a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp.

Zr

As sucrorested above, polyethylene glycol may be attached to proteins via linkage to any In r-I zn L] of a number of amino acid residues. For example, polyethylene erlycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, alutamic acid, or cysteine

Z--

residues. One or more reaction chemistries may. . .

accomplished by

any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless

systems for attaching polyethylene erlycol to

z1_Z1- C]

proteins are described in Del cyado et al., Crit. Rev. Thera. Drug Carrier. . .

One system for attaching polyethylene glycol directly to amino acid residues of proteins

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without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchlorlde (ClSO2CH2CF₃). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene (Flycol molecule having a .

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2,2,2-trifluorethane sulphonyl group.

Polyethylene glycol can also be attached to proteins usiner a number of different

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intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene crlycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1, I'-carbonylidimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitroph&noiccarbonate, and various. . .

protein of the invention

(i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 31 4, 5] 69 71 8, 91 10, 12, 15, 17, 20, or more polyethylene glycol. . .

heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation,

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iodination, methylation, myristylation, oxidation, peylation, proteolytic processing

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phosphorylation, . . .

the polynucleotide encoding this polypeptide. An Immunocrenic epitope, as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for creneratinic antibodies described

infra. (See, for example, Geysen et al., Proc. Nati. Acad. Sci. USA 81:3998-4002

(1983)). The term antigenic epitope, as used herein, is defined as a portion of a

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protein to which an antibody can immunospecifically bind its antigen as determined by

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any method well known in the art, for example, by the immunoassays described herein. . . .

85, 90, 95, or

100 amino acid residues in length. Antigenic epitopes are useful, for example, to raise

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bodies, including monoclonal antibodies, that specifically bind the epitope.

Non-limiting examples of antigenic polypeptides or peptides that can be used to

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generate TRIO receptor-specific antibodies include: a

polypeptide comprising amino acid

'due from about 57 to about 113 Figures I A-D (2 to 58 in SEQ. . . .

et al., J. Gen. Virol. 66:2347-2354 (1985). The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response

together with a carrier protein, such as an albumin, to an animal system (such as, for example, rabbit or mouse), or, . . . a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to

be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce

antibodies according to methods well known in the art

including, but not limited to, in vivo immunization, in vitro immunization, and . . . al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid.

For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as Zn in Z17 glutaraldehyde. Animals such as, for example, rabbits, rats, and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 micrograms of peptide or C! carrier protein and Freund's adjuvant. . . . Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody that can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface.

The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

chains of mammalian immunoglobulins. See, e.g., EP t] 1-In tn 394,827; Traunecker et al., Nature, 331:84-86 (1988). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing. . . .

detecting over-expression of TRIO, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors, for example. Assay techniques that can be used to determine levels of a protein, such as a TRIO protein of the. . . .

Preferred for assaying TRIO protein levels in a biological sample are antibody-based techniques. For example, TRIO protein expression in tissues can be studied with classical immunohistological methods. (M. Jalkanen et al., J. Cell. Biol. . . .

Jalkanen et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting TRIO receptor gene expression include immunoassays, such

as the enzyme linked
Z] Zn
immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ('I, 125i, . . .

Antibodies
The present invention further relates to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, preferably an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric bodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term antibody, as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site. . .

Most preferably the antibodies are human antigen-binding antibody fragments n t) in of the present invention and include, but are not limited to, Fab, FaV and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody tn framents, including single-chain antibodies, may comprise the variable region(s) Z] Z1_ L] alone or in combination with the entirety or a portion of the following: hincne. . . In fragments also comprising any combination of variable region(s) with a hincne region, Z] L] L_ in CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. As used LI ; n Z] herein, human antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin C 4n libraries or from animals transgenic for one or more human immunoglobulin and that

do not express endocrenous. . . .

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of. . . .

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the. . . herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies that specifically bind any epitope or polypeptide of the present invention may also be excluded.

Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, . . . known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less. . . .

Further included in the present invention are antibodies that bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention.. . . .

The invention also provides antibodies that competitively inhibit bindiner of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example,. . . .

In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at. . . .

Antibodies of the present invention may act as acyonists or antagonists of the C tn polypeptides of the present invention. For example, the present

invention includes

antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. The invention features both receptor-specific

antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation.

serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand or receptor activity by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as

L_nI_t
'bodies which bind the . . . I prevent the ligand from binding the receptor. Further the L_nI_t ion are 'bodies which activate the receptor. These antibodies may act as receptor agonists, anti I I I 1 e., potentiate or activate either all or a subset of the biological activities of the I'cyand mediated receptor activation. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities

4n t-I LI LI
of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen, et al., Cancer Res. 58(16):3668-3678 (1998)-1 Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon, et al., J. Immunol.

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the

antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g.

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Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response.

n tr

For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including but . . .

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention. . . administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include. . .

Monoclonal antibodies can be prepared using a wide variety of techniques

In known in the art including the use of hybridoma, recombinant, and phage display

L technologies, or a combination thereof. For example, monoclonal antibodies can be

In

produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hämmerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term monoclonal antibody as used herein is not limited to antibodies produced through hybridoma technology. The term monoclonal antibody refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it.

In Methods for producing and screening for specific antibodies using hybridoma technology are routine and well-known in the art and are discussed in detail in Example 6, below. Briefly, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested. In and splenocytes isolated. The splenocytes are and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments that recognize specific epitopes may be generated by Zn known techniques. For example, Fab and F(ab')2 fragments of the invention.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phage can be

I In utilized to display antigen-binding domains expressed from a repertoire or

L] combinatorial antibody library (e.g., human or murine). Phage expressing an antigen

t] C] L- binding domain that binds the antigen of interest can be. . . these methods are typically filamentous phage including fd and

Z- M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv

antibody domains recombinantly fused to either the phage crenes III or gene VIII

protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol.

Queen et al., U.S. Patent No. 5,585,089-, Riechmann et al., Nature 332:323 (1988), which are

incorporated herein by reference in their entireties.)

Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-(Yraftinc, (EP 239,400; PCT publication WO 91/09967; . . .

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin crenes. . . or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents

endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The

Zn r_1 transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all

or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by . . . and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of t7] ZI C this technology for producing human antibodies. see Lonberg and Hildszer (1995, Int.

Z 27] Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 96/34096; WO 96/33735; U.S. Patent Nos. 5,413,923; 5,625,126-1; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; and 5,939,598, . . . entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

L L] Zn Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as guided selection. In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that mimic polypeptides of the invention using 4n techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be. . . .

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Polynucleotides Encoding Antibodies
The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide

of the invention,
preferably, an antibody that binds to a polypeptide having the
amino acid sequence of
SEQ ID NO:2.

the nucleotide sequence of the
polynucleotides determined, by any method known in the art. For example,
if the
nucleotide sequence of the antibody is known, a polynucleotide
encoding the antibody
may be assembled from chemically synthesized oligonucleotides (e.g., as
described in
Kutmeler et al., BioTechniques 17:242 (1994)), which, briefly, involves
the synthesis
of. . .

In
Alternatively, a polynucleotide encoding an antibody may be
generated from
nucleic acid from a suitable source. If a clone containing a nucleic
acid encoding a
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particular antibody is not available, but the sequence of the
antibody molecule is
known, a nucleic acid encoding the immunoglobulin may be obtained from
a suitable
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source (e.g., an antibody cDNA library, or a cDNA library
generated from, or nucleic
preferably poly A+ RNA, isolated from, any tissue or cells expressing
the
acl

antibody, such as hybridoma cells selected to express an
antibody of the invention) by
PCR amplification using synthetic primers hybridizable to the 3' and
5' ends of the
sequence or by cloning using an. . . oligonucleotide probe specific
for the particular gene
n Zn
sequence to identify, e.g., a cDNA clone from a cDNA library that
encodes the
antibody. Amplified nucleic acids generated by PCR may then be
cloned into
replicable cloning vectors using any method well known in the. . .

Once the nucleotide sequence and corresponding amino acid sequence of
the
antibody is determined, the nucleotide sequence of the
antibody may be manipulated
using methods well known in the art for the manipulation of nucleotide
sequences,
e.g., recombinant DNA techniques, site directed. . . Protocols in
Molecular Biology, John
Wiley & Sons, NY, which are both incorporated by reference herein in
their entireties
, to generate antibodies having a different amino acid
sequence, for example to create
amino acid substitutions, deletions, and/or insertions.

for a listing of human framework
regions). Preferably, the polynucleotide generated by the combination
of the
framework regions and CDRs encodes an antibody that
specifically binds a

polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such In t:) methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to tn LI , regenerate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the. . .

In addition, techniques developed for the production of chimeric antibodies (Morrison et al., 1984, Proc. Nati. Acad. Sci. 81:851-855; Neuberger et al., 1984, Zn Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain Z-) antibodies (U.S. Patent No. 4,694,778; Bird, 1988, Science 242:423- 42; Huston et al., 1988, Proc. Nati. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-54) can be adapted to produce single chain antibodies. Single chain n tn antibodies are formed by linking the heavy and light chain fragments of the Fv region Z:1 L] via an amino acid bridge, resulting in a single chain. . .

Methods of Producing Antibodies
The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, e.g., a heavy or light chain of an antibody of the invention, requires construction of an expression vector containing a polynucleotide

that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the

production of the antibody molecule may be produced by recombinant DNA

technology using techniques well known in the art. Thus, methods for preparing a

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protein by expressing a polynucleotide containing an antibody encoding nucleotide

sequence are described herein. Methods which are well known to those skilled in the

art can be used to construct expression vectors containing antibody coding sequences

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and appropriate transcriptional and translational control signals. These methods

include, for example, in vitro recombinant DNA techniques, synthetic techniques, and

in vivo genetic recombination. The invention, thus, provides replicable vectors

comprising a nucleotide sequence encoding an antibody molecule of the invention, or

a heavy or light chain thereof, or a heavy or light chain variable domain, operably

linked to a promoter. Such vectors may include the nucleotide sequence encoding the

constant region of the antibody molecule (see, e.g., PC7 Publication WO 86/05807;

PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable

domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an

antibody of the invention. Thus, the invention includes host cells containing a

polynucleotide encoding an antibody of the invention, or a heavy or light chain

thereof, operably linked to a heterologous promoter. In preferred embodiments for the

expression of double-chained antibodies, vectors encoding both the heavy and light

chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, . . .

A variety of host-expression vector systems may be utilized to express the

antibody molecules of the invention. Such host-expression systems represent vehicles

by which the coding sequences of interest may be produced and subsequently purified,

but also represent cells which may, when transformed or transfected with the

appropriate nucleotide coding sequences, express an antibody molecule of the

invention in situ. These include but are not limited to microorganisms such as bacteria

(e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., *baculovirus*) containing antibody coding sequences, r-1 Z-- in plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., *Ti* plasmid) containing antibody coding t] zn sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) Z1- harbor in recombinant expression constructs containing promoters derived from. . .

In Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate. . .

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed.

example, when a large quantity of such a protein is to be produced, for the

In generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified

Zn may be desirable. Such. . . but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein. . .

californica nuclear polyhedrosis virus tn (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned

Zn Z-- individually into non-essential regions (for example the polyhedrin gene) of the virus

and placed under control. . . .

a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.cr., the late promoter and tripartite leader sequence. . . (e.cr., region tn Zn cl Zn E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.cr., see Logan & Shenk, 1984, Proc. Nati.

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Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the tn ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the. . . .

host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T35 W138, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HT132, BT20 and T47D, and normal mammary gland cell zn line such as, for example,

For longer-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA. . . .

can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule.

Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification. . . cloned genes in mammalian cells in DNA cloning, Vol (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker Crene. Since the amplified region is associated with the antibody crene, production of the antibody will t] In also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

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Once an antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification. . .

Antibody Conjugates

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention to create fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the Z' polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in. . .

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding. . . Fc: portions. Higher multimeric forms can be made by fusing the polypeptides to portions of Iota and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT. . .

Moreover, the antibodies or fragments thereof of the present invention can be

fused to marker sequences, such as a peptide to facilitates their purification. . . .

derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the flag tag C] 1-n

The present invention further encompasses antibodies or fragments thereof

conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment

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regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials,

radioactive materials, positron emitting. . . .

Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of. . . .

Further, an antibody or fragment thereof may be conjugated to a therapeutic

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moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or

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a radioactive metal ion.. . . acent that is

C) zn

detrimental to cells. Examples include paclitaxol, cytochalasin B, Orramycin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

(BSNU)

and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon,

B-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti- angiogenic aorent, . . .

Antibodies may also be attached to solid supports, which are particularly useful

I I for immunoassays or purification of the tarcret anti(yen. Such. . .

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.cr., Arnon et al., Monoclonal Antibodies For Immunotargeting Of

L t! Zn Drucrs In Cancer Therapy, in Monoclonal Antibodies And Cancer Therapy, Relsfeld

L] et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., Antibodies For Drua Delivery, in Controlled Drucr Delivery (2nd Ed.), Robinson et al. (eds.), pp.

In Zr 623-53 (Marcel Dekker, Inc. 1987); Thorpe, Antibody Carriers Of Cytotoxic Agents

Zn In Cancer Therapy: A Review, in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy, in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates, Immunol. Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an

g I antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems usiner techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), sandwich immunoassays, immunoprecipitation assays, precipitin reactions,

gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A. . . .

phosphate at pH 7.2, 1 % Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4°C, adding protein A 27. . . or more at 4°C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see,

or non-
In in fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking
In n Zn C the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing. . . .

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound, instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody

conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill.

Current

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Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at

The binding affinity of an antibody to an antigen and the off-rate of an

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antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^{3}H or ^{125}I) with the antibody of interest in the

Z-1 in

presence of increasing amounts of unlabeled antigen, and the detection of the antibody

Zln

bound to the labeled antigen. The affinity of the antibody of interest for a particular

C]

antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using

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radioimmunoassays. In this case, the antigen is incubated with antibody of interest is

conjugated to a labeled compound (e.g., ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

Antibody-Based Therapeutic Uses

The present invention is further directed to antibody-based therapies which

involve administering antibodies of the invention to an animal, preferably a mammal,

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and most preferably a human, patient for treating one or more of. . . to,

'bodies of the invention (including fragments, analogs and derivatives thereof as

anti I I Z' Zn

described herein) and nucleic acids encoding antibodies of the invention (including

fragments, analogs and derivatives thereof as described herein). The antibodies of the

invention can be used to treat, inhibit or prevent diseases and disorders associated

with aberrant expression and/or activity of a. . . and/or activity of a

polypeptide of the invention includes, but is not limited to, alleviating symptoms

associated with those diseases and disorders. Antibodies of the invention may be

included in pharmaceutically acceptable compositions as known in the art or as

provi I I 1

described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachinners provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e. g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered

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to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or

C]
neutralizing antibodies against polypeptides or polynucleotides of the present

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invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments

:r
thereof. Preferred binding affinities include those with a. . .

Antibody-Based Gene Therapy
In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity. . .

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors

zn zn

that express the antibody or fragments or chimeric proteins or heavy or light chains

thereof in a suitable host. In particular, such nucleic acid sequences have promoters

operably linked to the antibody coding region, said

promoter being inducible or

constitutive, and, optionally, tissue-specific. In another particular embodiment,

nucleic acid molecules are used in which the antibody coding sequences and any other

desired sequences are flanked by regions that promote homologous recombination at a

C in

desired site in the genome, thus providing for intrachromosomal expression of the

antibody nucleic acids (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA

86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438). In specific embodiments,

the expressed antibody molecule is a single chain

antibody; alternatively, the nucleic

acid sequences include sequences encoding both the heavy and light chains, or

fragments thereof, of the antibody.

or coating with lipids or cell-surface receptors or transfecting

zn In

agents, encapsulation in liposomes, microparticles, or microcapsules, or by

administering them in linkage to a peptide which is known to enter the nucleus, by

administering it in linkage to a ligand subject to receptor-mediated endocytosis (see,

e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used).

In a specific embodiment, viral vectors that contain nucleic acid sequences

encoding an antibody of the invention are used. For example, a retroviral vector can

be used (see Miller et al., 1993, Meth. Enzymol. 217:581-599)

that are not necessary for packaging of

the viral genome and integration into host cell DNA. The nucleic acid sequences

rn

encoding the antibody to be used in gene therapy are cloned into one or more vectors,

which facilitates delivery of the gene into a. . . .

In an embodiment in which recombinant cells are used in gene therapy, nucleic

Z]-

acid sequences encoding an antibody are introduced into the cells such that they are

expressible by the cells or their progeny, and the recombinant cells are. . . .

In a specific embodiment, the nucleic acid to be introduced for purposes of

gene therapy comprises an inducible promoter operably linked

to the codin CF re Gion,
t] zn
such that expression of the nucleic acid is controllable by controlling
the presence or
zn
absence. . .

Demonstration of Antibody-Based Therapeutic or Prophylactic
Activity

The compounds or pharmaceutical compositions of the invention are
preferably
tested in vitro, and then in vivo for the. . .

Antibody-Based Therapeutic Prophylactic Administration and
Composition

The invention provides methods of treatment, inhibition and prophylaxis
by
administration to a subject of an effective amount of a compound or
pharmaceutical
composition of the invention, preferably an antibody of the
invention. In a preferred
aspect, the compound is substantially purified (e.g., substantially free
from
substances that limit its effect or. . .

a porous, non-porous, or gelatinous material,
z1 tn
includincr membranes, such as sialastic membranes, or fibers.
Preferably, when
administering a protein, including an antibody, of the
invention, care must be taken to
use materials to which the protein does not absorb.

in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat
et
al., in Liposomes in the Therapy of Infectious Disease and
Cancer, Lopez-Berestem
and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein,
ibid., pp.

aun; Blolistic, Dupont), or coating
tn n C LI
with lipids or cell-surface receptors or transfectincr agents, or by
administerincr it in
:n t_1
linkacre to a horneobox- like peptide which is known to enter
the nucleus (see e.g.,
Jollot et al., 1991, Proc. Nati. Acad.. . .

For antibodies, the dosacye administered to a patient is
typically 0. 1 mg/kcr to
100 mcr/k(y of the patient's body weight. Preferably, the. . .
mcr/kcr to 10 mcy/ka of the patient's body weight. Generally, human
'bodies have a loncrer half-life within the human body than
antibodies from other
anti L]
species due to the immune response to the foreign polypeptides. Thus,
lower dosages
C] Z.,
of human antibodies and less frequent administration is often
possible. Further, the
dosage and frequency of administration of antibodies of the
invention may be reduced
rn

by enhanciner uptake and tissue penetration (e.g., into the brain) of the antibodies by C tn modifications such as, for example, lipidation.

Antibody-Based Diagnosis and Imaging
Labeled antibodies, and derivatives and analoas thereof, which specifically L]
bind to a polypeptide of interest can be used for diagnostic purposes to detect, zn diaunose, . . . interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual usincFone or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an. . .
assaying the expression of the polypeptide of interest in cells or body n Zn fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of crene expression with a standard cyene expression level, tn Z] L whereby. . . in the assayed polypeptide crene expression level t, compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively hi oh amount of transcript in blopsied L]
tissue from an individual may indicate a predisposition for. . . may allow health C y professionals to employ preventative measures or aaressive treatment earlier thereby preventing the development or further progression of the cancer

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistolocrical methods known to those. . . see Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., tn J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enz me linked Zn C] y immunoassorbent assay (ELISA) and the radtojmmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; C radioisotopes, such as iodine ('1, 125i, 123i, . . . a human subject, the In tn quantity of radioactivity injected will normally range from about 5 to 20 millicuries of

LI

99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., Immunopharmacokinetics of Radiolabeled

Antibodies and Their Fraoments. (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Antibody-Based Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially Isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound., or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diaonostic kit for use in screening serum containincy antibodies specific against proliferative

C] C LI and/or cancerous polynucleotides and polypeptides. Such a kit may include a control

antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide anticren antibody. Further, such a kit includes means for detecting the bindina of said antibody to the anticren (e.cr., the

antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may.

kit includes a solid support to which said polypeptide antigen is attached. Such a kit may

also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the reporter-labeled antibody.

for use in screening serum containing antigens of the polypeptide of the invention. The

t] Zn L diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the

antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove

L] L] Zn unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by.

or kit for carrying out this

Zn diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting

LI t) surface-bound anti-antigen antibody.

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Therapeutic Compositions and Methods

The Tumor Necrosis Factor (TNF) family ligands are known to be among the most

pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity,

In C Z]

anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (D.V. Goeddel et al., Tumor Necrosis Factors: Gene Structure and Biological Activities, Symp. Quant. Biol. 51:597-609 (1986), Cold Spring Harbor, B. Beutler and A.

for studying the phenotypic effects that result from inhibiting TRAIL/FR10 interactions on various cell types. TRIO

polypeptides and antagonists (e. g. monoclonal antibodies to TR IO) also may be used in in vitro assays for detecting TRAIL or TRIO or the interactions thereof.

The therapeutic compositions and methods described in this section include those

antibody-based composition and methods described in detail above. For example, the agonists and antagonists, and methods of using such agonists and antagonists, include the antibodies and their uses described above.

treated or prevented by the polynucleotides, polypeptides and/or agonists or antagonists

LI LI
of the invention include, but are not limited to, cancers (such as f0111CL11ar lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, allografts, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis. . . . rejection. In preferred embodiments, TRIO polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression,

Z., zn

and/or metastasis of cancers, in particular those listed above, or in the paragraph that follows.

and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endoteliellosarcoma, lymphangiomyoma, lymphangiosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland

carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, Juno carcinoma, small cell Juno carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma,

craniopharyngioma,
ependymoma, pinealoma, hemangioblastoma, acoustic neuroma,
oligodendroglioma,
Zn
menangioma, melanoma, neuroblastoma, . . .

invention

and agonists or antagonists thereof, are used to treat or prevent autoimmune diseases and/or inhibit the growth, progression, and/or metastasis of cancers, including, but not limited to, those cancers disclosed herein, such as, for example, lymphocytic leukemias (including, for example, MLL and chronic lymphocytic leukemia (CLL)) and follicular lymphomas. In another embodiment TRIO polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof, are used to activate, differentiate or proliferate cancerous cells or tissue (e.g., B cell lineage related cancers (e.g., CLL and MLL), lymphocytic leukemia, or lymphoma) and thereby render the cells more vulnerable to cancer therapy (e.g., chemotherapy

:n

or radiation therapy).

but

are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar

In

degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjögren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic. . . myocardial infarction, stroke and reperfusion injury), liver injury (such as hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. In preferred embodiments, TRIO polynucleotides, polypeptides and/or agonists. . .

Fas include, but are not limited to, soluble Fas polypeptides;

Z_n multimeric forms of soluble Fas polypeptides (e.g., dimers of sFas/Fc); anti-Fas antibodies

1-n

that bind Fas without transducing the biological signal that results in apoptosis; anti-Fas-ligand

Z_n I= tr

antibodies that block binding of Fas-ligand to Fas; and muteins of Fas-ligand that bind Fas but

C_n U

do not transduce the biological signal that results in apoptosis. Preferably, the antibodies

In

employed according to this method are monoclonal antibodies. Examples of suitable agents for

L_n zn

blocking Fas-ligand/Fas interactions, including blocking anti-Fas monoclonal antibodies, are
C LI zn
described in International application publication number WO 95/10540,
hereby incorporated
by reference.

receptor
'bodies that bind the TRAIL receptor without transducing the biolocrical signal that results in
anti I I I zn tn
apoptosis, anti-TRAIL antibodies that block bindina of TRAIL to one or more TRAIL receptors, and muteins of TRAIL that bind TRAIL receptors but do not transduce the biological signal that results in apoptosis. Preferably, the antibodies employed according to this method in
are monoclonal antibodies.

ancyiocyenesis is strinrently regulated and spatially and temporally delimited. Under conditions of pathological anglogenesis such as that characterizing
tr :n Z-- LI
solid tumor crrowth, these regulatory controls fall. Unregulated ancriocrenesis becomes
C LI LI tn Z71
pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor errowth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses et al., Biotech. 9:630-634 (199 I)-, Folkman et al., N. Engl. J. Mcd., 333:1757-1763 (1995); Auerbach et al., J. Microvasc. Res. 29:401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, Am. J. Ophthalmol. 94:715-743 (1982); and Folkman et al., . . .

4n zn
For example, significant data have accumulated which suggest that the growth of solid tumors
LI zn
is dependent on ancylocrenests. Folkman and Klagsbrun, Science 235:442-447 (1987).

L]
which can be treated with the polynucleotides and polypeptides of the invention include, but are not limited to those malianances, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman et al., Medicine, 2d Ed.,. . .

glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.cr reviews by Waltman et al., Am. J. Ophthal. 85:704-710 (1978) and. . .

of

blood composition-affecting disorders, such as, for example, hemophilia, cystic fibrosis, pregnancy, menstrual disorders, early anemia of prematurity, spinal cord injury, aGing,

In

various neoplastic disease states, and the like. Examples of patient conditions that require

supplementation of the oxygen carrying capacity of blood and which.

dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more

antibodies (e.pr., IcrG, I(YA, IcYM, and I(YE), to induce higher affinity antibody production (e.cr

L] t t Z:1 Z-, L

IgG, IcrA, IcrM, and IcrE), and/or to increase an immune response.

to an animal (Including, but not limited to, those listed above, and also

includincy transcrenic animals) incapable of producing functional endogenous antibody

t] Z-- tn

molecules or having an otherwise compromised endocFenous immune system, but which is

zn

capable of producing human immunocriobulin molecules by means. . .

An adjuvant to enhance tumor-specific immune responses.

As an auent to induce higher affinity antibodies.

As an auent to boost immunoresponsiveness amoncr B cell 'mmunodeficient individuals. B cell immunodeficiencies that may be ameliorated or treated by. . . or polynticleotides of the invention, or agoilists thereof, include, but are not limited to, SCID, conuenital a(yammaolobulinemia, common variable immunodeficiency, Wiskott-Aldrich Syndrome, X-linked immunodeficiency with hyper ICYM, and severe combined immunodeficiency.

in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonization of anti cren presentation may be useful as an anti-tumor treatment or to modulate the immune system.

As a means to induce tumor proliferation and thus make it more susceptible to anti-

neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus

Zn Z71

refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more

r]

their susceptibility profile would likely change.

As an anticFen for the aeneration of antibodies to inhibit or enhance TRIO mediated responses.

Antacronists of TRIO include binding and/or inhibitory

antibodies, antisense nucleic
ribozymes or soluble forms of the TRIO receptor(s) (e.g., the TRIO-Fc
molecule
aci
described in Example 38). These would be expected. . .

treatment or prevention of a wide range of diseases
and/or conditions. Such diseases and conditions include, but are not
limited to, cancer (e.g.,
immune cell related cancers, breast cancer, prostate
cancer, ovarian cancer, follicular
lymphoma, cancer associated with mutation or alteration of
p53, brain tumor, bladder cancer,
uterocervical cancer, colon cancer, colorectal
cancer, non-small cell carcinoma of the lung
small cell carcinoma of the lung, stomach cancer, etc.),
lymphoproliferative disorders (e.g.
t, t]-q
lymphadenopathy), microbial (e.g., viral, bacterial, etc.) infection
(e.g., HIV-1 infection,
r_1 LI
HIV-2 infection, herpesvirus infection (including, but. . .

decreased apoptosis or decreased cytokine and adhesion molecule
expression
is exhibited. An agonist can include soluble forms of TRIO and
monoclonal antibodies directed
against the TRIO polypeptide.

I]] I I in
increased apoptosis or NFkB expression is exhibited. An antagonist can
include soluble forms
of TRIO and monoclonal antibodies directed against the TRIO
polypeptide.

:n
Another screening technique well known in the art involves expressing in
cells a
]n
construct wherein the receptor is linked to a phospholipase C
or D. Exemplary cells include
endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The
screening may. . .

as, for example, TNF family ligand peptide fragments, transforming
growth
L- LI Z] Z]
factor, neurotransmitters (such as glutamate, dopamine, N-
methyl-D-aspartate), tumor
tn
suppressors (p53), cytolytic T cells and antimetabolites. Preferred
agonists include
Z!,
chemotherapeutic drugs such as, for example, cisplatin,
doxorubicin, bleomycin, cytosine
L]
arabinoside, nitroren mustard, methotrexate and vincristine. Others
include ethanol and -
1-n

arnyloid peptide. (Science 267:1457-1458 (1995)). Further preferred
agonists include
polyclonal and monoclonal antibodies raised against the TRIO
polypeptide, or a fragment
C :n

thereof. Such acronist antibodies raised acylnst a TNF-family receptor are disclosed in L.A.

Cowpox virus
crinA, Epstein-Barr virus BHRF], LMP-1, African swine fever Virus LMVV5-HL, and
Herpesvirus yl 34.5). calpain inhibitors, cysteine protease inhibitors, and tumor promoters
(such as PMA, Phenobarbital, and 0-Hexachlorocyclohexane).

L L r - In
(1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res. 5:539-549 (1988)). To this end,
the oligonucleotide may be conjugated to another molecule,
e.g., a peptide, hybridization

Z] Zn
triggered cross-linkiner auent, transport acent,
hybridization-trl(ycreder cleavacre acent, etc.

the cell surface bound forms of the receptor for

L In
bindincy to TNF-family ligands. Antagonists of the present invention also include antibodies

t]'
specific for TNF-family licrands and TRIO-Fc fusion proteins.

Montgomery et al., Eur. Cytokine Newt. 7:159 (1996). Further, antibodies specific for the extracellular domain of this block HSV-I entry into cells. Thus, TRIO antagonists of the present invention include both TRIO amino acid sequences and antibodies capable of preventing mediated viral entry into cells. Such sequences and antibodies can function by either competing with cell surface localized for binding to virus or by directly blocking binding

In
of virus to cell. . .

Antibodies accordincr to the present Invention may be prepared by any of a variety of methods usino TRIO immunogens of the present. . .

Polyclonal and monoclonal antibody aconists or antagonists accordiner to the present

Zn C] Zn
invention can be raised according to the methods disclosed herein and and/or. . .

addition, due to lymphoblast expression of TRIO, soluble TRIO agonist or zn antagonist mABs may be used to treat this form of cancer.

of the invention (see generally, Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in t:] tn
the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing TRIO polypeptide my be prepared by methods. . .

Chemotherapeutic agents that may be administered with the

t] zn
compositions of the invention include, but are not limited to,
antibiotic derivatives (e.g.,
doxorubicin, bleomycin, daunorubicin, and dactinomycin);
antiestrocrens (e.g., tamoxifen);
antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine,
interferon alpha-2b,
tr
glutamic acid, plicamycin, mercaptourine, and 6-thioguanine); cytotoxic.
.

In a specific embodiment, compositions of the invention are administered in
combination with CHOP (cyclophosphamide, doxorubicin,
vincristine, and prednisone) or
any combination of the components of CHOP. In another embodiment,
compositions of the
invention are administered in combination. . . .

Welch Medical Library. The relationship between Genes
In
and diseases that have been mapped to the same chromosomal region are
then identified
through linkage analysis (coinheritance of physically adjacent
genes).
.

wa
Z—Zn y
io as to produce that polypeptide with the six His residues (i.e., a 6 X
His tacy) covalently linked
In
to the carboxyl terminus of that polypeptide. However, in this example,
the polypeptide coding
sequence is inserted such that translation of the. . . .

a
polyadenylation signal arranged so that a cDNA conveniently can be
placed under expression
Z--
control of the CNIV promoter and operably linked to the SV40
intron and the polyadenylation
signal by means of restriction sites in the polylinker.
.

37:767 (1984). The fusion of
the HA tag to the target protein allows easy detection of the
recombinant protein with an
t] zn
antibody that recognizes the HA epitope.
.

Expression of the TRIO-FLA fusion protein is detected by radiolabelling
and
immunoprecipitation, using methods described in, for example Harlow et
al., Antibodies: a
Laboratory Manual. 2nd Ed., Cold Spring Harbor Laboratory Press, Cold
Spring Harbor, NY
L] In

(1988). To this end, two days after. . . . Wilson et al. cited
above. Proteins are precipitated from the cell lysate and from the
culture media using an HA-
specific monoclonal antibody. The precipitated proteins then
are analyzed by SDS-PAGE and
autoradiography. An expression product of the expected size is seen
in. . . .

C] L
the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is
tn t] t)
linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art
In
that this approach may be. . .

expressed soluble His-FLAG-tagged TRAIL. The resulting complex was
In Z
precipitated with protein G-Sepharose and bound TRAIL detected by Western blotting with anti-FLAG antibody. Like DR4, DR5, and TR5 (TRID), TRIO bound TRAIL. Corroborating this ability to bind TRAIL was the finding that TRIO-Fc, like. . .

is capable of -5 substantially attenuating TRAIL-induced cell death, suggesting that TRIO antagonizes TRAIL

Z7] C11 In L
signaling

L tn'

Example 6

Production of an Antibody

a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing TRIO are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of TRIO protein is prepared and purified to render it substantially free of natural contaminants. . .

Monoclonal antibodies specific for TRIO protein are prepared using hybridoma

rn technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol.

Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with TRIO polypeptide or, more. . .

et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the TRIO polypeptide.

Alternatively, additional antibodies capable of binding to TRIO polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is

C to obtain an antibody which binds to a second antibody . In accordance with possi 1

this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the TRIO protein-specific antibody can be blocked by TRIO.

Such antibodies comprise anti-Idiotypic antibodies to the TRIO protein-specific 'body and are used to immunize an animal to induce formation of further TRIO anti I protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is humanized. Such 'bodies can be produced using crenetic constructs derived from hybridoma cells anti I : t] producing the monoclonal antibodies described above. Methods for producing Z] tn chimeric and humanized antibodies are known in the art and are discussed infra. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); . . . al., WO 8601533; Robinson et al., WO 8702671; Boullanne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed Against TRIO From A Library Of scFvs L]

Naturally occurring V-crenes isolated from human PBLs are constructed into a

1-In I,, library of antibody fragments which contain reactivities a(Yainst TR IO to which the

In

donor may or may not have been exposed (see e.g., U.S.. . .

library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phacre displaying antibody fragments, approximately 109 E. coil harboring the phacremid are

Zn r_1 Z

used to inoculate 50 ml of 2xTY containing 1% glucose. . .

for precise mapping are

t] Z1_ L]

obtained usincr a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination

L] Zn

with a cooled charcre-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson, Cv. et al., Genet. Anal. Tech. Appl., 8:75 (1991).)

Image collection,. . .

For example, antibody-sandwich ELISAs are used to detect TRIO in a sample, preferably a biological sample. Wells of a microtiter plate are coated

with specific antibody
]) I I I I I ies to
TRIO, at a final concentration of 0.2 to 10 ucy/ml. The
antibodies are either monoclonal or
LI
polyclonal and are produced using technique known in the art. The wells
are blocked so that
r_1
binding of. . .

Next, 50 ul of specific antibody-alkaline phosphatase
conjugate, at a concentration of
I 0 25-400 ncr, is added and incubated for 2 hours at room temperature.
The plates are again
washed three times with delonized or distilled water to remove unbounded
conjugate.

Preferred antagonists for use in the present invention are TRIO-specific
antibodies.

Antisense technology is used to inhibit production of TRIO. This
technology is one
In n
example of a method of decreasing levels of TRIO polypeptide, preferably
a soluble and/or
secreted form, due to a variety of etiologies, such as cancer.

RNA) TRIO sequences into an animal
to increase or decrease the expression of the TRIO polypeptide. The TRIO
polynucleotide may
be operatively linked to a promoter or any other (Yenetic
elements necessary for the expression
of the TRIO polypeptide by the taraet tissue. Such. . .

flanking the promoter. The tarcreting sequence will be
Zn Zn
sufficiently near the 5'end of TRIO so the promoter will be operably
linked to the endoCFenous
Zn
sequence upon homologous recombination. The promoter and the targeting
sequences can be
r, r_1 Zn
amplified usiner PCR. Preferably, the. . .

Once the cells are transfected, homologous recombination will take place
which results
C)
in the promoter beiner operably linked to the endocrenous TRIO
sequence. This results in the
expression of TRIO in the cell. Expression may be detected by
immunological. . .

and 88% N,) tissue culture incubator, and after 7 days, analyzed for
expression of
differentiation antigens by staining with various monoclonal
antibodies and FACScan.

88% N 2)
L] rn
tissue culture incubator, and after 7 days, analyzed for expression of
differentiation antigens by
staining with various monoclonal antibodies and FACScan.

using a necrative selection procedure, where the committed cells of

most of the lineages are removed using a panel of monoclonal antibodies (anti cd I I b, CD4.

which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and C-1 in Zn

IL-15 synergize with SAC and IgM crosslinking to elicit. . .

Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of TRIO proteins.

PBS containing 1 % BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4°C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton.

PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4°C.

Vascular Endothelial Cells

For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-yl) (3-carboxymethoxyphenyl) (4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1. . .

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a. . .

Proliferating cell nuclear anticyclic (PCNA) in skin specimens is demonstrated by

In using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer served as a positive tissue control and human brain tissue is used as a negative tissue control.

Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8,. . .

was prepared that consists of a soluble form of TRIO (corresponding to amino acids -55 to 149 of SEQ ID NO:2) linked

to the Fc portion of a human
-5 Icrg I immuno(yloulin molecule. The ability of this protein to alter
the proliferative. . .

as priming acent and
In L- 1-In

Neutrokin-alpha as a second signal (data not shown). It is important to
note that other Tumor
Necrosis Factor Receptors (TNFR) fusion proteins (e.g., DR4-Fc
(Internatioaril Application
Publication No. WO 98/32856), TR6-Fc (Internatioaril Application
Publication No. WO
98/31799), and TR9-Fc. . .

ID NO:2; and a polypeptide
comprising amino acid residues from about 195 to about 228 in SEQ ID
NO:2
tn
22. An isolated antibody that binds specifically to a TRIO
receptor polypeptide of

CLMEN 28 The antibody of claim 22 that is an scFv fragment.
LI

29 The antibody of claim 22 that is an Fab fragment.

rn

Figure IA

10 30 50

CCACCC-%C:GCGTCCCCCACGCGTCCGGAGA,-A,CCTTTCvC-'%CCCGC.XCA]2%ACTACGGGC-AC
70 90 110

GAT=cTCATTGATTTGGCGC=CGATCC-A,CCCTCCTCCCTCTC.NTC-GGACTTTGG

130 150 170

GC-ACA,AAGCGTCCCCACCGCCTCGAGCGCTCGACCAGGC-CGCT-A.TCC.A.GGACCCAGGA.C-k
G P T A S - . . . S? T -1 z A C - - - ttjoL#v A T I I 5 I ali a IL =3
P75

NA i 7PIJL-R] a rmmml 3 0 HWAi 3 L Z LQ 9G &C L. IMQ E SHM T 111RAZZ L C.

. . ng/ml SCF

c

o0aft

c 0] + 1 ng/ml IL-3 and

E 5,000

5 n9iml SCF

2,500

low

.0 Le

La

TRI 0 (ng/ml)

SEQUENCE LISTING

<110> Human Genome Sciences, Inc.

<120> Human Tumor Necrosis Factor Receptor TR10

<130> PF379PCT2

<140> Unassigned

<141> 2000 25

<150> 60/136,786

<151> 1999 28

<150> 60/142,563

<151> 1999 07

<150> 60/144f023

<151> 1999 ILS

<160> 16

<170> PatentIn Ver.. . .